

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>7</sup> :</b> <b>C07H 21/04, C07K 1/00, 14/00, C12N 1/21, 15/00, 15/09, 15/63, 15/70, C12P 19/34</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 00/52027</b> <b>(43) International Publication Date:</b> 8 September 2000 (08.09.00)
<b>(21) International Application Number:</b> PCT/US00/05432 <b>(22) International Filing Date:</b> 2 March 2000 (02.03.00)  <b>(30) Priority Data:</b> 60/122,389 2 March 1999 (02.03.99) US 60/126,049 23 March 1999 (23.03.99) US 60/136,744 28 May 1999 (28.05.99) US  <b>(71) Applicant:</b> LIFE TECHNOLOGIES, INC. [US/US]; 9800 Medical Center Drive, Rockville, MD 20850 (US).  <b>(72) Inventors:</b> HARTLEY, James, L.; 7409 Hillside Drive, Frederick, MD 21702 (US). BRASCH, Michael, A.; 20931 Sunnycres Road, Gaithersburg, MD 20882 (US). TEMPLE, Gary, F.; 114 Ridge Road, Washington Grove, MD 20882 (US). CHEO, David; 2006 Baltimore Road, #21, Rockville, MD 20851 (US).  <b>(74) Agents:</b> ESMOND, Robert, W. et al.; Sterne, Kessler, Goldstein & Fox P.L.L.C., Suite 600, 1100 New York Avenue, N.W., Washington, DC 20005-3934 (US).	<b>(81) Designated States:</b> AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>With an indication in relation to deposited biological material furnished under Rule 13bis separately from the description.</i>	
<b>(54) Title:</b> COMPOSITIONS AND METHODS FOR USE IN RECOMBINATIONAL CLONING OF NUCLEIC ACIDS  <b>(57) Abstract</b>  The present invention relates generally to compositions and methods for use in recombinational cloning of nucleic acid molecules. In particular, the invention relates to nucleic acid molecules encoding one or more recombination sites or portions thereof, to nucleic acid molecules comprising one or more of these recombination site nucleotide sequences and optionally comprising one or more additional physical or functional nucleotide sequences. The invention also relates to vectors comprising the nucleic acid molecules of the invention, to host cells comprising the vectors or nucleic acid molecules of the invention, to methods of producing polypeptides using the nucleic acid molecules of the invention, and to polypeptides encoded by these nucleic acid molecules or produced by the methods of the invention. The invention also relates to antibodies that bind to one or more polypeptides of the invention or epitopes thereof. The invention also relates to the use of these compositions in methods for recombinational cloning of nucleic acids, <i>in vitro</i> and <i>in vivo</i> , to provide chimeric DNA molecules that have particular characteristics and/or DNA segments.		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav	TM	Turkmenistan
BF	Burkina Faso	GR	Greece		Republic of Macedonia	TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's	NZ	New Zealand		
CM	Cameroon		Republic of Korea	PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		



## Compositions and Methods for Use in Recombinational Cloning of Nucleic Acids

5

### BACKGROUND OF THE INVENTION

#### *Field of the Invention*

10

15

20

25

30

The present invention relates generally to recombinant DNA technology. More particularly, the present invention relates to compositions and methods for use in recombinational cloning of nucleic acid molecules. The invention relates specifically to nucleic acid molecules encoding one or more recombination sites or one or more partial recombination sites, particularly *attB*, *attP*, *attL*, and *attR*, and fragments, mutants, variants and derivatives thereof. The invention also relates to such nucleic acid molecules wherein the one or more recombination site nucleotide sequences is operably linked to the one or more additional physical or functional nucleotide sequences. The invention also relates to vectors comprising the nucleic acid molecules of the invention, to host cells comprising the vectors or nucleic acid molecules of the invention, to methods of producing polypeptides and RNAs encoded by the nucleic acid molecules of the invention, and to polypeptides encoded by these nucleic acid molecules or produced by the methods of the invention, which may be fusion proteins. The invention also relates to antibodies that bind to one or more polypeptides of the invention or epitopes thereof, which may be monoclonal or polyclonal antibodies. The invention also relates to the use of these nucleic acid molecules, vectors, polypeptides and antibodies in methods for recombinational cloning of nucleic acids, *in vitro* and *in vivo*, to provide chimeric DNA molecules that have particular characteristics and/or DNA segments. More particularly, the antibodies of the invention may be used to identify and/or purify proteins or fusion proteins encoded by the nucleic acid molecules or vectors of the invention, or to identify and/or purify the nucleic acid molecules of the invention.

*Related Art*

*Site-specific recombinases.* Site-specific recombinases are proteins that are present in many organisms (e.g. viruses and bacteria) and have been characterized to have both endonuclease and ligase properties. These recombinases (along with associated proteins in some cases) recognize specific sequences of bases in DNA and exchange the DNA segments flanking those segments. The recombinases and associated proteins are collectively referred to as "recombination proteins" (see, e.g., Landy, A., *Current Opinion in Biotechnology* 3:699-707 (1993)).

Numerous recombination systems from various organisms have been described. See, e.g., Hoess *et al.*, *Nucleic Acids Research* 14(6):2287 (1986); Abremski *et al.*, *J. Biol. Chem.* 261(1):391 (1986); Campbell, *J. Bacteriol.* 174(23):7495 (1992); Qian *et al.*, *J. Biol. Chem.* 267(11):7794 (1992); Araki *et al.*, *J. Mol. Biol.* 225(1):25 (1992); Maeser and Kahnmann *Mol. Gen. Genet.* 230:170-176 (1991); Esposito *et al.*, *Nucl. Acids Res.* 25(18):3605 (1997).

Many of these belong to the integrase family of recombinases (Argos *et al.* *EMBO J.* 5:433-440 (1986); Voziyanov *et al.*, *Nucl. Acids Res.* 27:930 (1999)). Perhaps the best studied of these are the Integrase/*att* system from bacteriophage  $\lambda$  (Landy, A. *Current Opinions in Genetics and Devel.* 3:699-707 (1993)), the Cre/*loxP* system from bacteriophage P1 (Hoess and Abremski (1990) In *Nucleic Acids and Molecular Biology*, vol. 4. Eds.: Eckstein and Lilley, Berlin-Heidelberg: Springer-Verlag; pp. 90-109), and the FLP/FRT system from the *Saccharomyces cerevisiae* 2  $\mu$  circle plasmid (Broach *et al.* *Cell* 29:227-234 (1982)).

Backman (U.S. Patent No. 4,673,640) discloses the *in vivo* use of  $\lambda$  recombinase to recombine a protein producing DNA segment by enzymatic site-specific recombination using wild-type recombination sites *attB* and *attP*.

Hasan and Szybalski (*Gene* 56:145-151 (1987)) discloses the use of  $\lambda$  Int recombinase *in vivo* for intramolecular recombination between wild type *attP* and *attB* sites which flank a promoter. Because the orientations of these sites are

inverted relative to each other, this causes an irreversible flipping of the promoter region relative to the gene of interest.

Palazzolo *et al.* *Gene* 88:25-36 (1990), discloses phage lambda vectors having bacteriophage  $\lambda$  arms that contain restriction sites positioned outside a cloned DNA sequence and between wild-type *loxP* sites. Infection of *E. coli* cells that express the Cre recombinase with these phage vectors results in recombination between the *loxP* sites and the *in vivo* excision of the plasmid replicon, including the cloned cDNA.

Pósfai *et al.* (*Nucl. Acids Res.* 22:2392-2398 (1994)) discloses a method for inserting into genomic DNA partial expression vectors having a selectable marker, flanked by two wild-type FRT recognition sequences. FLP site-specific recombinase as present in the cells is used to integrate the vectors into the genome at predetermined sites. Under conditions where the replicon is functional, this cloned genomic DNA can be amplified.

Bebee *et al.* (U.S. Patent No. 5,434,066) discloses the use of site-specific recombinases such as Cre for DNA containing two *loxP* sites for *in vivo* recombination between the sites.

Boyd (*Nucl. Acids Res.* 21:817-821 (1993)) discloses a method to facilitate the cloning of blunt-ended DNA using conditions that encourage intermolecular ligation to a dephosphorylated vector that contains a wild-type *loxP* site acted upon by a Cre site-specific recombinase present in *E. coli* host cells.

Waterhouse *et al.* (WO 93/19172 and *Nucleic Acids Res.* 21 (9):2265 (1993)) disclose an *in vivo* method where light and heavy chains of a particular antibody were cloned in different phage vectors between *loxP* and *loxP 511* sites and used to transfect new *E. coli* cells. Cre, acting in the host cells on the two parental molecules (one plasmid, one phage), produced four products in equilibrium: two different cointegrates (produced by recombination at either *loxP* or *loxP 511* sites), and two daughter molecules, one of which was the desired product.

Schlake & Bode (*Biochemistry* 33:12746-12751 (1994)) discloses an *in vivo* method to exchange expression cassettes at defined chromosomal locations, each flanked by a wild type and a spacer-mutated FRT recombination site. A

double-reciprocal crossover was mediated in cultured mammalian cells by using this FLP/FRT system for site-specific recombination.

Hartley *et al.* (U.S. Patent No. 5,888,732) disclose compositions and methods for recombinational exchange of nucleic acid segments and molecules, including for use in recombinational cloning of a variety of nucleic acid molecules *in vitro* and *in vivo*, using a combination of wildtype and mutated recombination sites and recombination proteins.

**Transposases.** The family of enzymes, the transposases, has also been used to transfer genetic information between replicons. Transposons are structurally variable, being described as simple or compound, but typically encode the recombinase gene flanked by DNA sequences organized in inverted orientations. Integration of transposons can be random or highly specific. Representatives such as Tn7, which are highly site-specific, have been applied to the *in vivo* movement of DNA segments between replicons (Lucklow *et al.*, *J. Virol.* 67:4566-4579 (1993)).

Devine and Boeke *Nucl. Acids Res.* 22:3765-3772 (1994), discloses the construction of artificial transposons for the insertion of DNA segments, *in vitro*, into recipient DNA molecules. The system makes use of the integrase of yeast TY1 virus-like particles. The DNA segment of interest is cloned, using standard methods, between the ends of the transposon-like element TY1. In the presence of the TY1 integrase, the resulting element integrates randomly into a second target DNA molecule.

**Recombination Sites.** Also key to the integration/recombination reactions mediated by the above-noted recombination proteins and/or transposases are recognition sequences, often termed "recombination sites," on the DNA molecules participating in the integration/recombination reactions. These recombination sites are discrete sections or segments of DNA on the participating nucleic acid molecules that are recognized and bound by the recombination proteins during the initial stages of integration or recombination. For example, the recombination site for Cre recombinase is *loxP* which is a 34 base pair sequence comprised of two 13 base pair inverted repeats (serving as the recombinase binding sites) flanking an 8 base pair core sequence. See Figure 1 of Sauer, B., *Curr. Opin. Biotech.*

5:521-527 (1994). Other examples of recognition sequences include the *attB*,  
*attP*, *attL*, and *attR* sequences which are recognized by the recombination protein  
 $\lambda$  Int. *attB* is an approximately 25 base pair sequence containing two 9 base pair  
core-type Int binding sites and a 7 base pair overlap region, while *attP* is an  
5 approximately 240 base pair sequence containing core-type Int binding sites and  
arm-type Int binding sites as well as sites for auxiliary proteins integration host  
factor (IHF), FIS and excisionase (Xis). See Landy, *Curr. Opin. Biotech.*  
3:699-707 (1993); see also U.S. Patent No. 5,888,732, which is incorporated by  
reference herein.

10 **DNA cloning.** The cloning of DNA segments currently occurs as a daily  
routine in many research labs and as a prerequisite step in many genetic analyses.  
The purpose of these clonings is various, however, two general purposes can be  
considered: (1) the initial cloning of DNA from large DNA or RNA segments  
(chromosomes, YACs, PCR fragments, mRNA, etc.), done in a relative handful  
15 of known vectors such as pUC, pGem, pBlueScript, and (2) the subcloning of  
these DNA segments into specialized vectors for functional analysis. A great deal  
of time and effort is expended both in the transfer of DNA segments from the  
initial cloning vectors to the more specialized vectors. This transfer is called  
subcloning.

20 The basic methods for cloning have been known for many years and have  
changed little during that time. A typical cloning protocol is as follows:

- (1) digest the DNA of interest with one or two restriction enzymes;
- (2) gel purify the DNA segment of interest when known;
- (3) prepare the vector by cutting with appropriate restriction  
25 enzymes, treating with alkaline phosphatase, gel purify etc., as  
appropriate;
- (4) ligate the DNA segment to the vector, with appropriate  
controls to eliminate background of uncut and self-ligated vector;
- (5) introduce the resulting vector into an *E. coli* host cell;
- 30 (6) pick selected colonies and grow small cultures overnight;
- (7) make DNA minipreps; and

(8) analyze the isolated plasmid on agarose gels (often after diagnostic restriction enzyme digestions) or by PCR.

The specialized vectors used for subcloning DNA segments are functionally diverse. These include but are not limited to: vectors for expressing nucleic acid molecules in various organisms; for regulating nucleic acid molecule expression; for providing tags to aid in protein purification or to allow tracking of proteins in cells; for modifying the cloned DNA segment (*e.g.*, generating deletions); for the synthesis of probes (*e.g.*, riboprobes); for the preparation of templates for DNA sequencing; for the identification of protein coding regions; for the fusion of various protein-coding regions; to provide large amounts of the DNA of interest, *etc.* It is common that a particular investigation will involve subcloning the DNA segment of interest into several different specialized vectors.

As known in the art, simple subclonings can be done in one day (*e.g.*, the DNA segment is not large and the restriction sites are compatible with those of the subcloning vector). However, many other subclonings can take several weeks, especially those involving unknown sequences, long fragments, toxic genes, unsuitable placement of restriction sites, high backgrounds, impure enzymes, *etc.* Subcloning DNA fragments is thus often viewed as a chore to be done as few times as possible.

Several methods for facilitating the cloning of DNA segments have been described, *e.g.*, as in the following references.

Ferguson, J., *et al. Gene* 16:191 (1981), discloses a family of vectors for subcloning fragments of yeast DNA. The vectors encode kanamycin resistance. Clones of longer yeast DNA segments can be partially digested and ligated into the subcloning vectors. If the original cloning vector conveys resistance to ampicillin, no purification is necessary prior to transformation, since the selection will be for kanamycin.

Hashimoto-Gotoh, T., *et al. Gene* 41:125 (1986), discloses a subcloning vector with unique cloning sites within a streptomycin sensitivity gene; in a streptomycin-resistant host, only plasmids with inserts or deletions in the dominant sensitivity gene will survive streptomycin selection.

Accordingly, traditional subcloning methods, using restriction enzymes and ligase, are time consuming and relatively unreliable. Considerable labor is expended, and if two or more days later the desired subclone can not be found among the candidate plasmids, the entire process must then be repeated with alternative conditions attempted. Although site specific recombinases have been used to recombine DNA *in vivo*, the successful use of such enzymes *in vitro* was expected to suffer from several problems. For example, the site specificities and efficiencies were expected to differ *in vitro*; topologically linked products were expected; and the topology of the DNA substrates and recombination proteins was expected to differ significantly *in vitro* (see, e.g., Adams *et al*, *J. Mol. Biol.* 226:661-73 (1992)). Reactions that could go on for many hours *in vivo* were expected to occur in significantly less time *in vitro* before the enzymes became inactive. In addition, the stabilities of the recombination enzymes after incubation for extended periods of time in *in vitro* reactions was unknown, as were the effects of the topologies (*i.e.*, linear, coiled, supercoiled, etc.) of the nucleic acid molecules involved in the reaction. Multiple DNA recombination products were expected in the biological host used, resulting in unsatisfactory reliability, specificity or efficiency of subcloning. Thus, *in vitro* recombination reactions were not expected to be sufficiently efficient to yield the desired levels of product.

Accordingly, there is a long felt need to provide an alternative subcloning system that provides advantages over the known use of restriction enzymes and ligases.

## SUMMARY OF THE INVENTION

The present invention relates to nucleic acid molecules encoding one or more recombination sites or one or more partial recombination sites, particularly *attB*, *attP*, *attL*, and *attR*, and fragments, mutants, variants and derivatives thereof. The invention also relates to such nucleic acid molecules comprising one or more of the recombination site nucleotide sequences or portions thereof and one or more additional physical or functional nucleotide sequences, such as those

encoding one or more multiple cloning sites, one or more transcription termination sites, one or more transcriptional regulatory sequences (*e.g.*, one or more promoters, enhancers, or repressors), one or more translational signal sequences, one or more nucleotide sequences encoding a fusion partner protein or peptide  
5 (*e.g.*, GST, His<sub>6</sub> or thioredoxin), one or more selection markers or modules, one or more nucleotide sequences encoding localization signals such as nuclear localization signals or secretion signals, one or more origins of replication, one or more protease cleavage sites, one or more desired proteins or peptides encoded by a gene or a portion of a gene, and one or more 5' or 3' polynucleotide tails  
10 (particularly a poly-G tail). The invention also relates to such nucleic acid molecules wherein the one or more recombination site nucleotide sequences is operably linked to the one or more additional physical or functional nucleotide sequences.

The invention also relates to primer nucleic acid molecules comprising the  
15 recombination site nucleotide sequences of the invention (or portions thereof), and to such primer nucleic acid molecules linked to one or more target-specific (*e.g.*, one or more gene-specific) primer nucleic acid sequences. Such primers may also comprise sequences complementary or homologous to DNA or RNA sequences to be amplified, *e.g.*, by PCR, RT-PCR, etc. Such primers may also comprise  
20 sequences or portions of sequences useful in the expression of protein genes (ribosome binding sites, localization signals, protease cleavage sites, repressor binding sites, promoters, transcription stops, stop codons, etc.). Said primers may also comprise sequences or portions of sequences useful in the manipulation of DNA molecules (restriction sites, transposition sites, sequencing primers, etc.).  
25 The primers of the invention may be used in nucleic acid synthesis and preferably are used for amplification (*e.g.*, PCR) of nucleic acid molecules. When the primers of the invention include target- or gene-specific sequences (any sequence contained within the target to be synthesized or amplified including translation signals, gene sequences, stop codons, transcriptional signals (*e.g.*, promoters) and  
30 the like), amplification or synthesis of target sequences or genes may be accomplished. Thus, the invention relates to synthesis of a nucleic acid molecules comprising mixing one or more primers of the invention with a nucleic acid



template, and incubating said mixture under conditions sufficient to make a first nucleic acid molecule complementary to all or a portion of said template. Thus, the invention relates specifically to a method of synthesizing a nucleic acid molecule comprising:

- (a) mixing a nucleic acid template with a polypeptide having polymerase activity and one or more primers comprising one or more recombination sites or portions thereof; and
- (b) incubating said mixture under conditions sufficient to synthesize a first nucleic acid molecule complementary to all or a portion of said template and which preferably comprises one or more recombination sites or portions thereof.

Such method of the invention may further comprise incubating said first synthesized nucleic acid molecule under conditions sufficient to synthesize a second nucleic acid molecule complementary to all or a portion of said first nucleic acid molecule. Such synthesis may provide for a first nucleic acid molecule having a recombination site or portion thereof at one or both of its termini.

In a preferred aspect, for the synthesis of the nucleic acid molecules, at least two primers are used wherein each primer comprises a homologous sequence at its terminus and/or within internal sequences of each primer (which may have a homology length of about 2 to about 500 bases, preferably about 3 to about 100 bases, about 4 to about 50 bases, about 5 to about 25 bases and most preferably about 6 to about 18 base overlap). In a preferred aspect, the first such primer comprises at least one target-specific sequence and at least one recombination site or portion thereof while the second primer comprises at least one recombination site or portion thereof. Preferably, the homologous regions between the first and second primers comprise at least a portion of the recombination site. In another aspect, the homologous regions between the first and second primers may comprise one or more additional sequences, *e.g.*, expression signals, translational start motifs, or other sequences adding functionality to the desired nucleic acid sequence upon amplification. In practice, two pairs of primers prime synthesis or amplification of a nucleic acid molecule. In a preferred aspect, all or at least a portion of the synthesized or amplified nucleic acid molecule will be homologous

to all or a portion of the template and further comprises a recombination site or a portion thereof at at least one terminus and preferably both termini of the synthesized or amplified molecule. Such synthesized or amplified nucleic acid molecule may be double stranded or single stranded and may be used in the recombination cloning methods of the invention. The homologous primers of the invention provide a substantial advantage in that one set of the primers may be standardized for any synthesis or amplification reaction. That is, the primers providing the recombination site sequences (without the target specific sequences) can be pre-made and readily available for use. This in practice allows the use of shorter custom made primers that contain the target specific sequence needed to synthesize or amplify the desired nucleic acid molecule. Thus, this provides reduced time and cost in preparing target specific primers (e.g., shorter primers containing the target specific sequences can be prepared and used in synthesis reactions). The standardized primers, on the other hand, may be produced in mass to reduce cost and can be readily provided (e.g., in kits or as a product) to facilitate synthesis of the desired nucleic acid molecules.

Thus, in one preferred aspect, the invention relates to a method of synthesizing or amplifying one or more nucleic acid molecules comprising:

- (a) mixing one or more nucleic acid templates with at least one polypeptide having polymerase or reverse transcriptase activity and at least a first primer comprising a template specific sequence (complementary to or capable of hybridizing to said templates) and at least a second primer comprising all or a portion of a recombination site wherein said at least a portion of said second primer is homologous to or complementary to at least a portion of said first primer; and
- (b) incubating said mixture under conditions sufficient to synthesize or amplify one or more nucleic acid molecules complementary to all or a portion of said templates and comprising one or more recombination sites or portions thereof at one and preferably both termini of said molecules.

More specifically, the invention relates to a method of synthesizing or amplifying one or more nucleic acid molecules comprising:

- 5 (a) mixing one or more nucleic acid templates with at least one polypeptide having polymerase or reverse transcriptase activity and at least a first primer comprising a template specific sequence (complementary to or capable of hybridizing to said templates) and at least a portion of a recombination site, and at least a second primer comprising all or a portion of a recombination site wherein said at least a portion of said recombination site on said second primer is complementary to or homologous to at least a portion of said recombination site on said first primer; and
- 10 (b) incubating said mixture under conditions sufficient to synthesize or amplify one or more nucleic acid molecules complementary to all or a portion of said templates and comprising one or more recombination sites or portions thereof at one and preferably both termini of said molecules.
- 15

In a more preferred aspect, the invention relates to a method of amplifying or synthesizing one or more nucleic acid molecules comprising:

- 20 (a) mixing one or more nucleic acid templates with at least one polypeptide having polymerase or reverse transcriptase activity and one or more first primers comprising at least a portion of a recombination site and a template specific sequence (complementary to or capable of hybridizing to said template);
- 25 (b) incubating said mixture under conditions sufficient to synthesize or amplify one or more first nucleic acid molecules complementary to all or a portion of said templates wherein said molecules comprise at least a portion of a recombination site at one and preferably both termini of said molecules;
- 30 (c) mixing said molecules with one or more second primers comprising one or more recombination sites, wherein said recombination sites of said second primers are homologous to or

complementary to at least a portion of said recombination sites on said first nucleic acid molecules; and

- (d) incubating said mixture under conditions sufficient to synthesize or amplify one or more second nucleic acid molecules complementary to all or a portion of said first nucleic acid molecules and which comprise one or more recombination sites at one and preferably both termini of said molecules.

The invention also relates to vectors comprising the nucleic acid molecules of the invention, to host cells comprising the vectors or nucleic acid molecules of the invention, to methods of producing polypeptides encoded by the nucleic acid molecules of the invention, and to polypeptides encoded by these nucleic acid molecules or produced by the methods of the invention, which may be fusion proteins. The invention also relates to antibodies that bind to one or more polypeptides of the invention or epitopes thereof, which may be monoclonal or polyclonal antibodies. The invention also relates to the use of these nucleic acid molecules, primers, vectors, polypeptides and antibodies in methods for recombinational cloning of nucleic acids, *in vitro* and *in vivo*, to provide chimeric DNA molecules that have particular characteristics and/or DNA segments.

The antibodies of the invention may have particular use to identify and/or purify peptides or proteins (including fusion proteins produced by the invention), and to identify and/or purify the nucleic acid molecules of the invention or portions thereof.

The methods for *in vitro* or *in vivo* recombinational cloning of nucleic acid molecule generally relate to recombination between at least a first nucleic acid molecule having at least one recombination site and a second nucleic acid molecule having at least one recombination site to provide a chimeric nucleic acid molecule. In one aspect, the methods relate to recombination between and first vector having at least one recombination site and a second vector having at least one recombination site to provide a chimeric vector. In another aspect, a nucleic acid molecule having at least one recombination site is combined with a vector having at least one recombination site to provide a chimeric vector. In a most preferred aspect, the nucleic acid molecules or vectors used in recombination

comprise two or more recombination sites. In a more specific embodiment of the invention, the recombination methods relate to a Destination Reaction (also referred to herein as an "LR reaction") in which recombination occurs between an Entry clone and a Destination Vector. Such a reaction transfers the nucleic acid molecule of interest from the Entry Clone into the Destination Vector to create an Expression Clone. The methods of the invention also specifically relate to an Entry or Gateward reaction (also referred to herein as a "BP reaction") in which an Expression Clone is recombined with a Donor vector to produce an Entry clone. In other aspects, the invention relates to methods to prepare Entry clones by combining an Entry vector with at least one nucleic acid molecule (e.g., gene or portion of a gene). The invention also relates to conversion of a desired vector into a Destination Vector by including one or more (preferably at least two) recombination sites in the vector of interest. In a more preferred aspect, a nucleic acid molecule (e.g., a cassette) having at least two recombination sites flanking a selectable marker (e.g., a toxic gene or a genetic element preventing the survival of a host cell containing that gene or element, and/or preventing replication, partition or heritability of a nucleic acid molecule (e.g., a vector or plasmid) comprising that gene or element) is added to the vector to make a Destination Vector of the invention.

Preferred vectors for use in the invention include prokaryotic vectors, eukaryotic vectors, or vectors which may shuttle between various prokaryotic and/or eukaryotic systems (e.g. shuttle vectors). Preferred prokaryotic vectors for use in the invention include but are not limited to vectors which may propagate and/or replicate in gram negative and/or gram positive bacteria, including bacteria of the genera *Escherichia*, *Salmonella*, *Proteus*, *Clostridium*, *Klebsiella*, *Bacillus*, *Streptomyces*, and *Pseudomonas* and preferably in the species *E. coli*. Eukaryotic vectors for use in the invention include vectors which propagate and/or replicate in yeast cells, plant cells, mammalian cells, (particularly human and mouse), fungal cells, insect cells, nematode cells, fish cells and the like. Particular vectors of interest include but are not limited to cloning vectors, sequencing vectors, expression vectors, fusion vectors, two-hybrid vectors, gene therapy vectors, phage display vectors, gene-targeting vectors, PACs, BACs, YACs, MACs, and

reverse two-hybrid vectors. Such vectors may be used in prokaryotic and/or eukaryotic systems depending on the particular vector.

In another aspect, the invention relates to kits which may be used in carrying out the methods of the invention, and more specifically relates to cloning or subcloning kits and kits for carrying out the LR Reaction (*e.g.*, making an Expression Clone), for carrying out the BP Reaction (*e.g.*, making an Entry Clone), and for making Entry Clone and Destination Vector molecules of the invention. Such kits may comprise a carrier or receptacle being compartmentalized to receive and hold therein any number of containers. Such containers may contain any number of components for carrying out the methods of the invention or combinations of such components. In particular, a kit of the invention may comprise one or more components (or combinations thereof) selected from the group consisting of one or more recombination proteins or auxiliary factors or combinations thereof, one or more compositions comprising one or more recombination proteins or auxiliary factors or combinations thereof (for example, GATEWAY™ LR Clonase™ Enzyme Mix or GATEWAY™ BP Clonase™ Enzyme Mix), one or more reaction buffers, one or more nucleotides, one or more primers of the invention, one or more restriction enzymes, one or more ligases, one or more polypeptides having polymerase activity (*e.g.*, one or more reverse transcriptases or DNA polymerases), one or more proteinases (*e.g.*, proteinase K or other proteinases), one or more Destination Vector molecules, one or more Entry Clone molecules, one or more host cells (*e.g.* competent cells, such as *E. coli* cells, yeast cells, animal cells (including mammalian cells, insect cells, nematode cells, avian cells, fish cells, etc.), plant cells, and most particularly *E. coli* DB3.1 host cells, such as *E. coli* LIBRARY EFFICIENCY® DB3.1™ Competent Cells), instructions for using the kits of the invention (*e.g.*, to carry out the methods of the invention), and the like. In related aspects, the kits of the invention may comprise one or more nucleic acid molecules encoding one or more recombination sites or portions thereof, particularly one or more nucleic acid molecules comprising a nucleotide sequence encoding the one or more recombination sites or portions thereof of the invention. Preferably, such nucleic acid molecules comprise at least two recombination sites which flank a selectable

marker (*e.g.*, a toxic gene and/or antibiotic resistance gene). In a preferred aspect, such nucleic acid molecules are in the form of a cassette (*e.g.*, a linear nucleic acid molecule comprising one or more and preferably two or more recombination sites or portions thereof).

5           Kits for inserting or adding recombination sites to nucleic acid molecules of interest may comprise one or more nucleases (preferably restriction endonucleases), one or more ligases, one or more topoisomerases, one or more polymerases, and one or more nucleic acid molecules or adapters comprising one or more recombination sites. Kits for integrating recombination sites into one or  
10           more nucleic acid molecules of interest may comprise one or more components (or combinations thereof) selected from the group consisting of one or more integration sequences comprising one or more recombination sites. Such integration sequences may comprise one or more transposons, integrating viruses, homologous recombination sequences, RNA molecules, one or more host cells  
15           and the like.

          Kits for making the Entry Clone molecules of the invention may comprise any or a number of components and the composition of such kits may vary depending on the specific method involved. Such methods may involve inserting the nucleic acid molecules of interest into an Entry or Donor Vector by the  
20           recombinational cloning methods of the invention, or using conventional molecular biology techniques (*e.g.*, restriction enzyme digestion and ligation). In a preferred aspect, the Entry Clone is made using nucleic acid amplification or synthesis products. Kits for synthesizing Entry Clone molecules from amplification or synthesis products may comprise one or more components (or combinations  
25           thereof) selected from the group consisting of one or more Donor Vectors (*e.g.*, one or more attP vectors including, but not limited to, pDONR201 (Figure 49), pDONR202 (Figure 50), pDONR203 (Figure 51), pDONR204 (Figure 52), pDONR205 (Figure 53), pDONR206 (Figure 53), and the like), one or more polypeptides having polymerase activity (preferably DNA polymerases and most preferably thermostable DNA polymerases), one or more proteinases, one or more  
30           reaction buffers, one or more nucleotides, one or more primers comprising one or

more recombination sites or portions thereof, and instructions for making one or more Entry Clones.

Kits for making the Destination vectors of the invention may comprise any number of components and the compositions of such kits may vary depending on the specific method involved. Such methods may include the recombination methods of the invention or conventional molecular biology techniques (e.g., restriction endonuclease digestion and ligation). In a preferred aspect, the Destination vector is made by inserting a nucleic acid molecule comprising at least one recombination site (or portion thereof) of the invention (preferably a nucleic acid molecule comprising at least two recombination sites or portions thereof flanking a selectable marker) into a desired vector to convert the desired vector into a Destination vector of the invention. Such kits may comprise at least one component (or combinations thereof) selected from the group consisting of one or more restriction endonucleases, one or more ligases, one or more polymerases, one or more nucleotides, reaction buffers, one or more nucleic acid molecules comprising at least one recombination site or portion thereof (preferably at least one nucleic acid molecule comprising at least two recombination sites flanking at least one selectable marker, such as a cassette comprising at least one selectable marker such as antibiotic resistance genes and/or toxic genes), and instructions for making such Destination vectors.

The invention also relates to kits for using the antibodies of the invention in identification and/or isolation of peptides and proteins (which may be fusion proteins) produced by the nucleic acid molecules of the invention, and for identification and/or isolation of the nucleic acid molecules of the invention or portions thereof. Such kits may comprise one or more components (or combination thereof) selected from the group consisting of one or more antibodies of the invention, one or more detectable labels, one or more solid supports and the like.

Other preferred embodiments of the present invention will be apparent to one of ordinary skill in light of what is known in the art, in light of the following drawings and description of the invention, and in light of the claims.



## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts one general method of the present invention, wherein the starting (parent) DNA molecules can be circular or linear. The goal is to exchange the new subcloning vector D for the original cloning vector B. It is desirable in one embodiment to select for AD and against all the other molecules, including the Cointegrate. The square and circle are sites of recombination: *e.g.*, *lox* (such as *loxP*) sites, *att* sites, *etc.* For example, segment D can contain expression signals, protein fusion domains, new drug markers, new origins of replication, or specialized functions for mapping or sequencing DNA. It should be noted that the cointegrate molecule contains Segment D (Destination vector) adjacent to segment A (Insert), thereby juxtaposing functional elements in D with the insert in A. Such molecules can be used directly in vitro (*e.g.*, if a promoter is positioned adjacent to a gene-for in vitro transcription/translation) or in vivo (following isolation in a cell capable of propagating *ccdB*-containing vectors) by selecting for the selection markers in Segments B+D. As one skilled in the art will recognize, this single step method has utility in certain envisioned applications of the invention.

Figure 2 is a more detailed depiction of the recombinational cloning system of the invention, referred to herein as the "GATEWAY™ Cloning System." This figure depicts the production of Expression Clones via a "Destination Reaction," which may also be referred to herein as an "LR Reaction." A *kan*<sup>r</sup> vector (referred to herein as an "Entry clone") containing a DNA molecule of interest (*e.g.*, a gene) localized between an *attL1* site and an *attL2* site is reacted with an *amp*<sup>r</sup> vector (referred to herein as a "Destination Vector") containing a toxic or "death" gene localized between an *attR1* site and an *attR2* site, in the presence of GATEWAY™ LR Clonase™ Enzyme Mix (a mixture of Int, IHF and Xis). After incubation at 25°C for about 60 minutes, the reaction yields an *amp*<sup>r</sup> Expression Clone containing the DNA molecule of interest localized between an *attB1* site and an *attB2* site, and a *kan*<sup>r</sup> byproduct molecule, as well as intermediates. The reaction mixture may then be transformed into host cells (*e.g.*, *E. coli*) and clones containing the nucleic acid molecule of interest may

be selected by plating the cells onto ampicillin-containing media and picking amp<sup>r</sup> colonies.

**Figure 3** is a schematic depiction of the cloning of a nucleic acid molecule from an Entry clone into multiple types of Destination vectors, to produce a variety of Expression Clones. Recombination between a given Entry clone and different types of Destination vectors (not shown), via the LR Reaction depicted in Figure 2, produces multiple different Expression Clones for use in a variety of applications and host cell types.

**Figure 4** is a detailed depiction of the production of Entry Clones via a "BP reaction," also referred to herein as an "Entry Reaction" or a "Gateward Reaction." In the example shown in this figure, an amp<sup>r</sup> expression vector containing a DNA molecule of interest (*e.g.*, a gene) localized between an *attB1* site and an *attB2* site is reacted with a kan<sup>r</sup> Donor vector (*e.g.*, an attP vector; here, GATEWAY™ pDONR201 (see Figure 49A-C)) containing a toxic or "death" gene localized between an *attP1* site and an *attP2* site, in the presence of GATEWAY™ BP Clonase™ Enzyme Mix (a mixture of Int and IHF). After incubation at 25°C for about 60 minutes, the reaction yields a kan<sup>r</sup> Entry clone containing the DNA molecule of interest localized between an *attL1* site and an *attL2* site, and an amp<sup>r</sup> by-product molecule. The Entry clone may then be transformed into host cells (*e.g.*, *E. coli*) and clones containing the Entry clone (and therefore the nucleic acid molecule of interest) may be selected by plating the cells onto kanamycin-containing media and picking kan<sup>r</sup> colonies. Although this figure shows an example of use of a kan<sup>r</sup> Donor vector, it is also possible to use Donor vectors containing other selection markers, such as the gentamycin resistance or tetracycline resistance markers, as discussed herein.

**Figure 5** is a more detailed schematic depiction of the LR ("Destination") reaction (Figure 5A) and the BP ("Entry" or "Gateward") reaction (Figure 5B) of the GATEWAY™ Cloning System, showing the reactants, products and byproducts of each reaction.

**Figure 6** shows the sequences of the attB1 and attB2 sites flanking a gene of interest after subcloning into a Destination Vector to create an Expression Clone.

**Figure 7** is a schematic depiction of four ways to make Entry Clones using the compositions and methods of the invention: 1. using restriction enzymes and ligase; 2. starting with a cDNA library prepared in an attL Entry Vector; 3. using an Expression Clone from a library prepared in an attB Expression Vector via the BxP reaction; and 4. recombinational cloning of PCR fragments with terminal attB sites, via the BxP reaction. Approaches 3 and 4 rely on recombination with a Donor vector (here, an attP vector such as pDONR201 (see Figure 49A-C), pDONR202 (see Figure 50A-C), pDONR203 (see Figure 51A-C), pDONR204 (see Figure 52A-C), pDONR205 (see Figure 53A-C), or pDONR206 (see Figure 54A-C), for example) that provides an Entry Clone carrying a selection marker such as kan<sup>r</sup>, gen<sup>r</sup>, tet<sup>r</sup>, or the like.

**Figure 8** is a schematic depiction of cloning of a PCR product by a BxP (Entry or Gateward) reaction. A PCR product with 25 bp terminal attB sites (plus four Gs) is shown as a substrate for the BxP reaction. Recombination between the attB-PCR product of a gene and a Donor vector (which donates an Entry Vector that carries kan<sup>r</sup>) results in an Entry Clone of the PCR product.

**Figure 9** is a listing of the nucleotide sequences of the recombination sites designated herein as attB1, attB2, attP1, attP2, attL1, attL2, attR1 and attR2. Sequences are written conventionally, from 5' to 3'.

**Figures 10-20:** The plasmid backbone for all the Entry Vectors depicted herein is the same, and is shown in Figure 10A for the Entry Vector pENTR1A. For other Entry Vectors shown in Figures 11-20, only the sequences shown in Figure "A" for each figure set (*i.e.*, Figure 11A, Figure 12A, etc.) are different (within the attL1-attL2 cassettes) from those shown in Figure 10 -- the plasmid backbone is identical.

**Figure 10** is a schematic depiction of the physical map and cloning sites (Figure 10A), and the nucleotide sequence (Figure 10B), of the Entry Vector pENTR1A.

**Figure 11** is a schematic depiction of the cloning sites (Figure 11A) and the nucleotide sequence (Figure 11B) of the Entry Vector pENTR2B.

**Figure 12** is a schematic depiction of the cloning sites (Figure 12A) and the nucleotide sequence (Figure 12B) of the Entry Vector pENTR3C.

5       **Figure 13** is a schematic depiction of the cloning sites (Figure 13A) and the nucleotide sequence (Figure 13B) of the Entry Vector pENTR4.

**Figure 14** is a schematic depiction of the cloning sites (Figure 14A) and the nucleotide sequence (Figure 14B) of the Entry Vector pENTR5.

10       **Figure 15** is a schematic depiction of the cloning sites (Figure 15A) and the nucleotide sequence (Figure 15B) of the Entry Vector pENTR6.

**Figure 16** is a schematic depiction of the cloning sites (Figure 16A) and the nucleotide sequence (Figure 16B) of the Entry Vector pENTR7.

**Figure 17** is a schematic depiction of the cloning sites (Figure 17A) and the nucleotide sequence (Figure 17B) of the Entry Vector pENTR8.

15       **Figure 18** is a schematic depiction of the cloning sites (Figure 18A) and the nucleotide sequence (Figure 18B) of the Entry Vector pENTR9.

**Figure 19** is a schematic depiction of the cloning sites (Figure 19A) and the nucleotide sequence (Figure 19B) of the Entry Vector pENTR10.

20       **Figure 20** is a schematic depiction of the cloning sites (Figure 20A) and the nucleotide sequence (Figure 20B) of the Entry Vector pENTR11.

25       **Figure 21** is a schematic depiction of the physical map and the Trc expression cassette (Figure 21A) showing the promoter sequences at -35 and at -10 from the initiation codon, and the nucleotide sequence (Figure 21B-D), of Destination Vector pDEST1. This vector may also be referred to as pTrc-DEST1.

30       **Figure 22** is a schematic depiction of the physical map and the His6 expression cassette (Figure 22A) showing the promoter sequences at -35 and at -10 from the initiation codon, and the nucleotide sequence (Figure 22B-D), of Destination Vector pDEST2. This vector may also be referred to as pHis6-DEST2.

**Figure 23** is a schematic depiction of the physical map and the GST expression cassette (Figure 23A) showing the promoter sequences at -35 and at -10 from the initiation codon, and the nucleotide sequence (Figure 23B-D), of Destination Vector pDEST3. This vector may also be referred to as pGST-DEST3.

**Figure 24** is a schematic depiction of the physical map and the His6-Trx expression cassette (Figure 24A) showing the promoter sequences at -35 and at -10 from the initiation codon and a TEV protease cleavage site, and the nucleotide sequence (Figure 24B-D), of Destination Vector pDEST4. This vector may also be referred to as pTrx-DEST4.

**Figure 25** is a schematic depiction of the attR1 and attR2 sites (Figure 25A), the physical map (Figure 25B), and the nucleotide sequence (Figure 25C-D), of Destination Vector pDEST5. This vector may also be referred to as pSPORT(+)-DEST5.

**Figure 26** is a schematic depiction of the attR1 and attR2 sites (Figure 26A), the physical map (Figure 26B), and the nucleotide sequence (Figure 26C-D), of Destination Vector pDEST6. This vector may also be referred to as pSPORT(-)-DEST6.

**Figure 27** is a schematic depiction of the attR1 site, CMV promoter, and the physical map (Figure 27A), and the nucleotide sequence (Figure 27B-C), of Destination Vector pDEST7. This vector may also be referred to as pCMV-DEST7.

**Figure 28** is a schematic depiction of the attR1 site, baculovirus polyhedrin promoter, and the physical map (Figure 28A), and the nucleotide sequence (Figure 28B-D), of Destination Vector pDEST8. This vector may also be referred to as pFastBac-DEST8.

**Figure 29** is a schematic depiction of the attR1 site, Semliki Forest Virus promoter, and the physical map (Figure 29A), and the nucleotide sequence (Figure 29B-E), of Destination Vector pDEST9. This vector may also be referred to as pSFV-DEST9.

**Figure 30** is a schematic depiction of the attR1 site, baculovirus polyhedrin promoter, His6 fusion domain, and the physical map (Figure 30A), and the nucleotide sequence (Figure 30B-D), of Destination Vector pDEST10. This vector may also be referred to as pFastBacHT-DEST10.

5        **Figure 31** is a schematic depiction of the attR1 cassette containing a tetracycline-regulated CMV promoter and the physical map (Figure 31A), and the nucleotide sequence (Figure 31B-D), of Destination Vector pDEST11. This vector may also be referred to as pTet-DEST11.

10        **Figure 32** is a schematic depiction of the attR1 site, the start of the mRNA of the CMV promoter, and the physical map (Figure 32A), and the nucleotide sequence (Figure 32B-D), of Destination Vector pDEST12.2. This vector may also be referred to as pCMVneo-DEST12, as pCMV-DEST12, or as pDEST12.

15        **Figure 33** is a schematic depiction of the attR1 site, the  $\lambda P_L$  promoter, and the physical map (Figure 33A), and the nucleotide sequence (Figure 33B-C), of Destination Vector pDEST13. This vector may also be referred to as p $\lambda P_L$ -DEST13.

20        **Figure 34** is a schematic depiction of the attR1 site, the T7 promoter, and the physical map (Figure 34A), and the nucleotide sequence (Figure 34B-D), of Destination Vector pDEST14. This vector may also be referred to as pPT7-DEST14.

25        **Figure 35** is a schematic depiction of the attR1 site, the T7 promoter, and the N-terminal GST fusion sequence, and the physical map (Figure 35A), and the nucleotide sequence (Figure 35B-D), of Destination Vector pDEST15. This vector may also be referred to as pT7 GST-DEST15.

30        **Figure 36** is a schematic depiction of the attR1 site, the T7 promoter, and the N-terminal thioredoxin fusion sequence, and the physical map (Figure 36A), and the nucleotide sequence (Figure 36B-D), of Destination Vector pDEST16. This vector may also be referred to as pT7 Trx-DEST16.

**Figure 37** is a schematic depiction of the attR1 site, the T7 promoter, and the N-terminal His6 fusion sequence, and the physical map (Figure 37A), and the

nucleotide sequence (Figure 37B-D), of Destination Vector pDEST17. This vector may also be referred to as pT7 His-DEST17.

5 **Figure 38** is a schematic depiction of the attR1 site and the p10 baculovirus promoter, and the physical map (Figure 38A), and the nucleotide sequence (Figure 38B-D), of Destination Vector pDEST18. This vector may also be referred to as pFBp10-DEST18.

10 **Figure 39** is a schematic depiction of the attR1 site, and the 39k baculovirus promoter, and the physical map (Figure 39A), and the nucleotide sequence (Figure 39B-D), of Destination Vector pDEST19. This vector may also be referred to as pFB39k-DEST19.

**Figure 40** is a schematic depiction of the attR1 site, the *polh* baculovirus promoter, and the N-terminal GST fusion sequence, and the physical map (Figure 40A), and the nucleotide sequence (Figure 40B-D), of Destination Vector pDEST20. This vector may also be referred to as pFB GST-DEST20.

15 **Figure 41** is a schematic depiction of a 2-hybrid vector with a DNA-binding domain, the attR1 site, and the ADH promoter, and the physical map (Figure 41A), and the nucleotide sequence (Figure 41B-E), of Destination Vector pDEST21. This vector may also be referred to as pDB Leu-DEST21.

20 **Figure 42** is a schematic depiction of a 2-hybrid vector with an activation domain, the attR1 site, and the ADH promoter, and the physical map (Figure 42A), and the nucleotide sequence (Figure 42B-D), of Destination Vector pDEST22. This vector may also be referred to as pPC86-DEST22.

25 **Figure 43** is a schematic depiction of the attR1 and attR2 sites, the T7 promoter, and the C-terminal His6 fusion sequence, and the physical map (Figure 43A), and the nucleotide sequence (Figure 43B-D), of Destination Vector pDEST23. This vector may also be referred to as pC-term-His6-DEST23.

30 **Figure 44** is a schematic depiction of the attR1 and attR2 sites, the T7 promoter, and the C-terminal GST fusion sequence, and the physical map (Figure 44A), and the nucleotide sequence (Figure 44B-D), of Destination Vector pDEST24. This vector may also be referred to as pC-term-GST-DEST24.

**Figure 45** is a schematic depiction of the attR1 and attR2 sites, the T7 promoter, and the C-terminal thioredoxin fusion sequence, and the physical map (Figure 45A), and the nucleotide sequence (Figure 45B-D), of Destination Vector pDEST25. This vector may also be referred to as pC-term-Trx-DEST25.

**Figure 46** is a schematic depiction of the attR1 site, the CMV promoter, and an N-terminal His6 fusion sequence, and the physical map (Figure 46A), and the nucleotide sequence (Figure 46B-D), of Destination Vector pDEST26. This vector may also be referred to as pCMV-SPneo-His-DEST26.

**Figure 47** is a schematic depiction of the attR1 site, the CMV promoter, and an N-terminal GST fusion sequence, and the physical map (Figure 47A), and the nucleotide sequence (Figure 47B-D), of Destination Vector pDEST27. This vector may also be referred to as pCMV-SPneo-GST-DEST27.

**Figure 48** is a depiction of the physical map (Figure 48A), the cloning sites (Figure 48B), and the nucleotide sequence (Figure 48C-D), for the attB cloning vector plasmid pEXP501. This vector may also be referred to equivalently herein as pCMV•SPORT6, pCMVSPORT6, and pCMVSport6.

**Figure 49** is a depiction of the physical map (Figure 49A), and the nucleotide sequence (Figure 49B-C), for the Donor plasmid pDONR201 which donates a kanamycin-resistant vector in the BP Reaction. This vector may also be referred to as pAttPkanr Donor Plasmid, or as pAttPkan Donor Plasmid

**Figure 50** is a depiction of the physical map (Figure 50A), and the nucleotide sequence (Figure 50B-C), for the Donor plasmid pDONR202 which donates a kanamycin-resistant vector in the BP Reaction.

**Figure 51** is a depiction of the physical map (Figure 51A), and the nucleotide sequence (Figure 51B-C), for the Donor plasmid pDONR203 which donates a kanamycin-resistant vector in the BP Reaction.

**Figure 52** is a depiction of the physical map (Figure 52A), and the nucleotide sequence (Figure 52B-C), for the Donor plasmid pDONR204 which donates a kanamycin-resistant vector in the BP Reaction.



**Figure 53** is a depiction of the physical map (Figure 53A), and the nucleotide sequence (Figure 53B-C), for the Donor plasmid pDONR205 which donates a tetracycline-resistant vector in the BP Reaction.

**Figure 54** is a depiction of the physical map (Figure 54A), and the nucleotide sequence (Figure 54B-C), for the Donor plasmid pDONR206 which donates a gentamycin-resistant vector in the BP Reaction. This vector may also be referred to as pENTR22 attP Donor Plasmid, pAttPGenr Donor Plasmid, or pAttPgnt Donor Plasmid.

**Figure 55** depicts the attB1 site, and the physical map, of an Entry Clone (pENTR7) of CAT subcloned into the Destination Vector pDEST2 (Figure 22).

**Figure 56** depicts the DNA components of Reaction B of the one-tube BxP reaction described in Example 16, pEZC7102 and attB-tet-PCR.

**Figure 57** is a physical map of the desired product of Reaction B of the one-tube BxP reaction described in Example 16, tetx7102.

**Figure 58** is a physical map of the Destination Vector pEZC8402.

**Figure 59** is a physical map of the expected tet<sup>r</sup> subclone product, tetx8402, resulting from the LxR Reaction with tetx7102 (Figure 57) plus pEZC8402 (Figure 58).

**Figure 60** is a schematic depiction of the bacteriophage lambda recombination pathways in *E. coli*.

**Figure 61** is a schematic depiction of the DNA molecules participating in the LR Reaction. Two different co-integrates form during the LR Reaction (only one of which is shown here), depending on whether attL1 and attR1 or attL2 and attR2 are first to recombine. In one aspect, the invention provides directional cloning of a nucleic acid molecule of interest, since the recombination sites react with specificity (attL1 reacts with attR1; attL2 with attR2; attB1 with attP1; and attB2 with attP2). Thus, positioning of the sites allows construction of desired vectors having recombined fragments in the desired orientation.

**Figure 62** is a depiction of native and fusion protein expression using the recombinational cloning methods and compositions of the invention. In the upper figure depicting native protein expression, all of the translational start signals are

included between the attB1 and attB2 sites; therefore, these signals must be present in the starting Entry Clone. The lower figure depicts fusion protein expression (here showing expression with both N-terminal and C-terminal fusion tags so that ribosomes read through attB1 and attB2 to create the fusion protein). Unlike native protein expression vectors, N-terminal fusion vectors have their translational start signals upstream of the attB1 site.

**Figure 63** is a schematic depiction of three GATEWAY™ Cloning System cassettes. Three blunt-ended cassettes are depicted which convert standard expression vectors to Destination Vectors. Each of the depicted cassettes provides amino-terminal fusions in one of three possible reading frames, and each has a distinctive restriction cleavage site as shown.

**Figure 64** shows the physical maps of plasmids containing three attR reading frame cassettes, pEZC15101 (reading frame A; Figure 64A), pEZC15102 (reading frame B; Figure 64B), and pEZC15103 (reading frame C; Figure 64C).

**Figure 65** depicts the attB primers used for amplifying the tet<sup>r</sup> and amp<sup>r</sup> genes from pBR322 by the cloning methods of the invention.

**Figure 66** is a table listing the results of recombinational cloning of the tet<sup>r</sup> and amp<sup>r</sup> PCR products made using the primers shown in Figure 65.

**Figure 67** is a graph showing the effect of the number of guanines (G's) contained on the 5' end of the PCR primers on the cloning efficiency of PCR products. It is noted, however, that other nucleotides besides guanine (including A, T, C, U or combinations thereof) may be used as 5' extensions on the PCR primers to enhance cloning efficiency of PCR products.

**Figure 68** is a graph showing a titration of various amounts of attP and attB reactants in the BxP reaction, and the effects on cloning efficiency of PCR products.

**Figure 69** is a series of graphs showing the effects of various weights (Figure 69A) or moles (Figure 69B) of a 256 bp PCR product on formation of colonies, and on efficiency of cloning of the 256 bp PCR product into a Donor Vector (Figure 69C).

**Figure 70** is a series of graphs showing the effects of various weights (Figure 70A) or moles (Figure 70B) of a 1 kb PCR product on formation of colonies, and on efficiency of cloning of the 1 kb PCR product into a Donor Vector (Figure 70C).

5 **Figure 71** is a series of graphs showing the effects of various weights (Figure 71A) or moles (Figure 71B) of a 1.4 kb PCR product on formation of colonies, and on efficiency of cloning of the 1.4 kb PCR product into a Donor Vector (Figure 71C).

10 **Figure 72** is a series of graphs showing the effects of various weights (Figure 72A) or moles (Figure 72B) of a 3.4 kb PCR product on formation of colonies, and on efficiency of cloning of the 3.4 kb PCR product into a Donor Vector (Figure 72C).

15 **Figure 73** is a series of graphs showing the effects of various weights (Figure 73A) or moles (Figure 73B) of a 4.6 kb PCR product on formation of colonies, and on efficiency of cloning of the 4.6 kb PCR product into a Donor Vector (Figure 73C).

**Figure 74** is photograph of an ethidium bromide-stained gel of a titration of a 6.9 kb PCR product in a BxP reaction.

20 **Figure 75** is a graph showing the effects of various amounts of a 10.1 kb PCR product on formation of colonies upon cloning of the 10.1 kb PCR product into a Donor Vector.

**Figure 76** is photograph of an ethidium bromide-stained gel of a titration of a 10.1 kb PCR product in a BxP reaction.

25 **Figure 77** is a table summarizing the results of the PCR product cloning efficiency experiments depicted in Figures 69-74, for PCR fragments ranging in size from 0.256 kb to 6.9 kb.

30 **Figure 78** is a depiction of the sequences at the ends of attR Cassettes. Sequences contributed by the  $Cm^r$ -ccdB cassette are shown, including the outer ends of the flanking attR sites (boxed). The staggered cleavage sites for Int are indicated in the boxed regions. Following recombination with an Entry Clone, only the outer sequences in attR sites contribute to the resulting attB sites in the

Expression Clone. The underlined sequences at both ends dictate the different reading frames (reading frames A, B, or C, with two alternative reading frame C cassettes depicted) for fusion proteins.

**Figure 79** is a depiction of several different attR cassettes (in reading frames A, B, or C) which may provide fusion codons at the amino-terminus of the encoded protein.

**Figure 80** illustrates the single-cutting restriction sites in an attR reading frame A cassette of the invention.

**Figure 81** illustrates the single-cutting restriction sites in an attR reading frame B cassette of the invention.

**Figure 82** illustrates the single-cutting restriction sites in two alternative attR reading frame C cassettes of the invention (Figures 82A and 82B) depicted in Figure 78.

**Figure 83** shows the physical map (Figure 83A), and the nucleotide sequence (Figure 83B-C), for an attR reading frame C parent plasmid prfC Parent III, which contains an attR reading frame C cassette of the invention (alternative A in Figures 78 and 82).

**Figure 84** is a physical map of plasmid pEZC1301.

**Figure 85** is a physical map of plasmid pEZC1313.

**Figure 86** is a physical map of plasmid pEZ14032.

**Figure 87** is a physical map of plasmid pMAB58.

**Figure 88** is a physical map of plasmid pMAB62.

**Figure 89** is a depiction of a synthesis reaction using two pairs of homologous primers of the invention.

**Figure 90** is a schematic depiction of the physical map (Figure 90A), and the nucleotide sequence (Figure 90B-D), of Destination Vector pDEST28.

**Figure 91** is a schematic depiction of the physical map (Figure 91A), and the nucleotide sequence (Figure 91B-D), of Destination Vector pDEST29.

**Figure 92** is a schematic depiction of the physical map (Figure 92A), and the nucleotide sequence (Figure 92B-D), of Destination Vector pDEST30.

**Figure 93** is a schematic depiction of the physical map (Figure 93A), and the nucleotide sequence (Figure 93B-D), of Destination Vector pDEST31.

**Figure 94** is a schematic depiction of the physical map (Figure 94A), and the nucleotide sequence (Figure 94B-E), of Destination Vector pDEST32.

5 **Figure 95** is a schematic depiction of the physical map (Figure 95A), and the nucleotide sequence (Figure 95B-D), of Destination Vector pDEST33.

**Figure 96** is a schematic depiction of the physical map (Figure 96A), and the nucleotide sequence (Figure 96B-D), of Destination Vector pDEST34.

10 **Figure 97** is a depiction of the physical map (Figure 97A), and the nucleotide sequence (Figure 97B-C), for the Donor plasmid pDONR207 which donates a gentamycin-resistant vector in the BP Reaction.

**Figure 98** is a schematic depiction of the physical map (Figure 98A), and the nucleotide sequence (Figure 98B-D), of the 2-hybrid vector pMAB85.

15 **Figure 99** is a schematic depiction of the physical map (Figure 99A), and the nucleotide sequence (Figure 99B-D), of the 2-hybrid vector pMAB86.

## DETAILED DESCRIPTION OF THE INVENTION

### 20 *Definitions*

In the description that follows, a number of terms used in recombinant DNA technology are utilized extensively. In order to provide a clear and consistent understanding of the specification and claims, including the scope to be given such terms, the following definitions are provided.

25 **Byproduct:** is a daughter molecule (a new clone produced after the second recombination event during the recombinational cloning process) lacking the segment which is desired to be cloned or subcloned.

30 **Cointegrate:** is at least one recombination intermediate nucleic acid molecule of the present invention that contains both parental (starting) molecules. It will usually be linear. In some embodiments it can be circular. RNA and polypeptides may be expressed from cointegrates using an appropriate host cell strain, for example *E. coli* DB3.1 (particularly *E. coli* LIBRARY EFFICIENCY®).

DB3.1™ Competent Cells), and selecting for both selection markers found on the cointegrate molecule.

**Host:** is any prokaryotic or eukaryotic organism that can be a recipient of the recombinational cloning Product, vector, or nucleic acid molecule of the invention. A "host," as the term is used herein, includes prokaryotic or eukaryotic organisms that can be genetically engineered. For examples of such hosts, *see* Maniatis *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (1982).

**Insert or Inserts:** include the desired nucleic acid segment or a population of nucleic acid segments (segment *A* of Figure 1) which may be manipulated by the methods of the present invention. Thus, the terms Insert(s) are meant to include a particular nucleic acid (preferably DNA) segment or a population of segments. Such Insert(s) can comprise one or more nucleic acid molecules.

**Insert Donor:** is one of the two parental nucleic acid molecules (e.g. RNA or DNA) of the present invention which carries the Insert. The Insert Donor molecule comprises the Insert flanked on both sides with recombination sites. The Insert Donor can be linear or circular. In one embodiment of the invention, the Insert Donor is a circular DNA molecule and further comprises a cloning vector sequence outside of the recombination signals (see Figure 1). When a population of Inserts or population of nucleic acid segments are used to make the Insert Donor, a population of Insert Donors results and may be used in accordance with the invention. Examples of such Insert Donor molecules are GATEWAY™ Entry Vectors, which include but are not limited to those Entry Vectors depicted in Figures 10-20, as well as other vectors comprising a gene of interest flanked by one or more *attL* sites (e.g., *attL1*, *attL2*, etc.), or by one or more *attB* sites (e.g., *attB1*, *attB2*, etc.) for the production of library clones.

**Product:** is one of the desired daughter molecules comprising the *A* and *D* sequences which is produced after the second recombination event during the recombinational cloning process (see Figure 1). The Product contains the nucleic acid which was to be cloned or subcloned. In accordance with the invention, when a population of Insert Donors are used, the resulting population of Product

molecules will contain all or a portion of the population of Inserts of the Insert Donors and preferably will contain a representative population of the original molecules of the Insert Donors.

**Promoter:** is a DNA sequence generally described as the 5'-region of a gene, located proximal to the start codon. The transcription of an adjacent DNA segment is initiated at the promoter region. A repressible promoter's rate of transcription decreases in response to a repressing agent. An inducible promoter's rate of transcription increases in response to an inducing agent. A constitutive promoter's rate of transcription is not specifically regulated, though it can vary under the influence of general metabolic conditions.

**Recognition sequence:** Recognition sequences are particular sequences which a protein, chemical compound, DNA, or RNA molecule (*e.g.*, restriction endonuclease, a modification methylase, or a recombinase) recognizes and binds. In the present invention, a recognition sequence will usually refer to a recombination site. For example, the recognition sequence for Cre recombinase is *loxP* which is a 34 base pair sequence comprised of two 13 base pair inverted repeats (serving as the recombinase binding sites) flanking an 8 base pair core sequence. See Figure 1 of Sauer, B., *Current Opinion in Biotechnology* 5:521-527 (1994). Other examples of recognition sequences are the *attB*, *attP*, *attL*, and *attR* sequences which are recognized by the recombinase enzyme  $\lambda$  Integrase. *attB* is an approximately 25 base pair sequence containing two 9 base pair core-type Int binding sites and a 7 base pair overlap region. *attP* is an approximately 240 base pair sequence containing core-type Int binding sites and arm-type Int binding sites as well as sites for auxiliary proteins integration host factor (IHF), FIS and excisionase (Xis). See Landy, *Current Opinion in Biotechnology* 3:699-707 (1993). Such sites may also be engineered according to the present invention to enhance production of products in the methods of the invention. When such engineered sites lack the P1 or H1 domains to make the recombination reactions irreversible (*e.g.*, *attR* or *attP*), such sites may be designated *attR'* or *attP'* to show that the domains of these sites have been modified in some way.

**Recombination proteins:** include excisive or integrative proteins, enzymes, co-factors or associated proteins that are involved in recombination reactions involving one or more recombination sites, which may be wild-type proteins (See Landy, *Current Opinion in Biotechnology* 3:699-707 (1993)), or mutants, derivatives (e.g., fusion proteins containing the recombination protein sequences or fragments thereof), fragments, and variants thereof.

**Recombination site:** is a recognition sequence on a DNA molecule participating in an integration/recombination reaction by the recombinational cloning methods of the invention. Recombination sites are discrete sections or segments of DNA on the participating nucleic acid molecules that are recognized and bound by a site-specific recombination protein during the initial stages of integration or recombination. For example, the recombination site for Cre recombinase is *loxP* which is a 34 base pair sequence comprised of two 13 base pair inverted repeats (serving as the recombinase binding sites) flanking an 8 base pair core sequence. See Figure 1 of Sauer, B., *Curr. Opin. Biotech.* 5:521-527 (1994). Other examples of recognition sequences include the *attB*, *attP*, *attL*, and *attR* sequences described herein, and mutants, fragments, variants and derivatives thereof, which are recognized by the recombination protein  $\lambda$  Int and by the auxiliary proteins integration host factor (IHF), FIS and excisionase (Xis). See Landy, *Curr. Opin. Biotech.* 3:699-707 (1993).

**Recombinational Cloning:** is a method described herein, whereby segments of nucleic acid molecules or populations of such molecules are exchanged, inserted, replaced, substituted or modified, *in vitro* or *in vivo*. By “*in vitro*” and “*in vivo*” herein is meant recombinational cloning that is carried out outside of host cells (e.g., in cell-free systems) or inside of host cells (e.g., using recombination proteins expressed by host cells), respectively.

**Repression cassette:** is a nucleic acid segment that contains a repressor or a Selectable marker present in the subcloning vector.

**Selectable marker:** is a DNA segment that allows one to select for or against a molecule (e.g., a replicon) or a cell that contains it, often under particular conditions. These markers can encode an activity, such as, but not limited to,



production of RNA, peptide, or protein, or can provide a binding site for RNA, peptides, proteins, inorganic and organic compounds or compositions and the like. Examples of Selectable markers include but are not limited to: (1) DNA segments that encode products which provide resistance against otherwise toxic compounds (e.g., antibiotics); (2) DNA segments that encode products which are otherwise lacking in the recipient cell (e.g., tRNA genes, auxotrophic markers); (3) DNA segments that encode products which suppress the activity of a gene product; (4) DNA segments that encode products which can be readily identified (e.g., phenotypic markers such as  $\beta$ -galactosidase, green fluorescent protein (GFP), and cell surface proteins); (5) DNA segments that bind products which are otherwise detrimental to cell survival and/or function; (6) DNA segments that otherwise inhibit the activity of any of the DNA segments described in Nos. 1-5 above (e.g., antisense oligonucleotides); (7) DNA segments that bind products that modify a substrate (e.g. restriction endonucleases); (8) DNA segments that can be used to isolate or identify a desired molecule (e.g. specific protein binding sites); (9) DNA segments that encode a specific nucleotide sequence which can be otherwise non-functional (e.g., for PCR amplification of subpopulations of molecules); (10) DNA segments, which when absent, directly or indirectly confer resistance or sensitivity to particular compounds; (11) DNA segments that encode products which are toxic in recipient cells; (12) DNA segments that inhibit replication, partition or heritability of nucleic acid molecules that contain them; and/or (13) DNA segments that encode conditional replication functions, e.g., replication in certain hosts or host cell strains or under certain environmental conditions (e.g., temperature, nutritional conditions, etc.).

**Selection scheme:** is any method which allows selection, enrichment, or identification of a desired Product or Product(s) from a mixture containing an Entry Clone or Vector, a Destination Vector, a Donor Vector, an Expression Clone or Vector, any intermediates (e.g. a Cointegrate or a replicon), and/or Byproducts. The selection schemes of one preferred embodiment have at least two components that are either linked or unlinked during recombinational cloning. One component is a Selectable marker. The other component controls the expression *in vitro* or *in vivo* of the Selectable marker, or survival of the cell (or

the nucleic acid molecule, *e.g.*, a replicon) harboring the plasmid carrying the Selectable marker. Generally, this controlling element will be a repressor or inducer of the Selectable marker, but other means for controlling expression or activity of the Selectable marker can be used. Whether a repressor or activator is used will depend on whether the marker is for a positive or negative selection, and the exact arrangement of the various DNA segments, as will be readily apparent to those skilled in the art. A preferred requirement is that the selection scheme results in selection of or enrichment for only one or more desired Products. As defined herein, selecting for a DNA molecule includes (a) selecting or enriching for the presence of the desired DNA molecule, and (b) selecting or enriching against the presence of DNA molecules that are not the desired DNA molecule.

In one embodiment, the selection schemes (which can be carried out in reverse) will take one of three forms, which will be discussed in terms of Figure 1. The first, exemplified herein with a Selectable marker and a repressor therefore, selects for molecules having segment *D* and lacking segment *C*. The second selects against molecules having segment *C* and for molecules having segment *D*. Possible embodiments of the second form would have a DNA segment carrying a gene toxic to cells into which the *in vitro* reaction products are to be introduced. A toxic gene can be a DNA that is expressed as a toxic gene product (a toxic protein or RNA), or can be toxic in and of itself. (In the latter case, the toxic gene is understood to carry its classical definition of "heritable trait".)

Examples of such toxic gene products are well known in the art, and include, but are not limited to, restriction endonucleases (*e.g.*, *DpnI*), apoptosis-related genes (*e.g.* ASK1 or members of the *bcl-2/ced-9* family), retroviral genes including those of the human immunodeficiency virus (HIV), defensins such as NP-1, inverted repeats or paired palindromic DNA sequences, bacteriophage lytic genes such as those from  $\Phi$ X174 or bacteriophage T4; antibiotic sensitivity genes such as *rpsL*, antimicrobial sensitivity genes such as *pheS*, plasmid killer genes, eukaryotic transcriptional vector genes that produce a gene product toxic to bacteria, such as GATA-1, and genes that kill hosts in the absence of a suppressing function, *e.g.*, *kicB*, *ccdB*,  $\Phi$ X174 *E* (Liu, Q. *et al.*, *Curr. Biol.*

8:1300-1309 (1998)), and other genes that negatively affect replicon stability and/or replication. A toxic gene can alternatively be selectable *in vitro*, e.g., a restriction site.

5 Many genes coding for restriction endonucleases operably linked to inducible promoters are known, and may be used in the present invention. *See*, e.g. U.S. Patent Nos. 4,960,707 (*DpnI* and *DpnII*); 5,000,333, 5,082,784 and 5,192,675 (*KpnI*); 5,147,800 (*NgoAIII* and *NgoAI*); 5,179,015 (*FspI* and *HaeIII*); 5,200,333 (*HaeII* and *TaqI*); 5,248,605 (*HpaII*); 5,312,746 (*ClaI*); 5,231,021 and 5,304,480 (*XhoI* and *XhoII*); 5,334,526 (*AluI*); 5,470,740 (*NsiI*); 5,534,428  
10 (*SstI/SacI*); 5,202,248 (*NcoI*); 5,139,942 (*NdeI*); and 5,098,839 (*PacI*). *See also* Wilson, G.G., *Nucl. Acids Res.* 19:2539-2566 (1991); and Lunnen, K.D., *et al.*, *Gene* 74:25-32 (1988).

In the second form, segment *D* carries a Selectable marker. The toxic gene would eliminate transformants harboring the Vector Donor, Cointegrate, and  
15 Byproduct molecules, while the Selectable marker can be used to select for cells containing the Product and against cells harboring only the Insert Donor.

The third form selects for cells that have both segments *A* and *D* in *cis* on the same molecule, but not for cells that have both segments in *trans* on different molecules. This could be embodied by a Selectable marker that is split into two  
20 inactive fragments, one each on segments *A* and *D*.

The fragments are so arranged relative to the recombination sites that when the segments are brought together by the recombination event, they reconstitute a functional Selectable marker. For example, the recombinational event can link a promoter with a structural nucleic acid molecule (e.g., a gene),  
25 can link two fragments of a structural nucleic acid molecule, or can link nucleic acid molecules that encode a heterodimeric gene product needed for survival, or can link portions of a replicon.

**Site-specific recombinase:** is a type of recombinase which typically has at least the following four activities (or combinations thereof): (1) recognition of  
30 one or two specific nucleic acid sequences; (2) cleavage of said sequence or sequences; (3) topoisomerase activity involved in strand exchange; and (4) ligase

activity to reseal the cleaved strands of nucleic acid. See Sauer, B., *Current Opinions in Biotechnology* 5:521-527 (1994). Conservative site-specific recombination is distinguished from homologous recombination and transposition by a high degree of sequence specificity for both partners. The strand exchange mechanism involves the cleavage and rejoining of specific DNA sequences in the absence of DNA synthesis (Landy, A. (1989) *Ann. Rev. Biochem.* 58:913-949).

**Subcloning vector:** is a cloning vector comprising a circular or linear nucleic acid molecule which includes preferably an appropriate replicon. In the present invention, the subcloning vector (segment *D* in Figure 1) can also contain functional and/or regulatory elements that are desired to be incorporated into the final product to act upon or with the cloned DNA Insert (segment *A* in Figure 1). The subcloning vector can also contain a Selectable marker (preferably DNA).

**Vector:** is a nucleic acid molecule (preferably DNA) that provides a useful biological or biochemical property to an Insert. Examples include plasmids, phages, autonomously replicating sequences (ARS), centromeres, and other sequences which are able to replicate or be replicated *in vitro* or in a host cell, or to convey a desired nucleic acid segment to a desired location within a host cell. A Vector can have one or more restriction endonuclease recognition sites at which the sequences can be cut in a determinable fashion without loss of an essential biological function of the vector, and into which a nucleic acid fragment can be spliced in order to bring about its replication and cloning. Vectors can further provide primer sites, *e.g.*, for PCR, transcriptional and/or translational initiation and/or regulation sites, recombinational signals, replicons, Selectable markers, *etc.* Clearly, methods of inserting a desired nucleic acid fragment which do not require the use of homologous recombination, transpositions or restriction enzymes (such as, but not limited to, UDG cloning of PCR fragments (U.S. Patent No. 5,334,575, entirely incorporated herein by reference), T:A cloning, and the like) can also be applied to clone a fragment into a cloning vector to be used according to the present invention. The cloning vector can further contain one or more selectable markers suitable for use in the identification of cells transformed with the cloning vector.

**Vector Donor:** is one of the two parental nucleic acid molecules (e.g. RNA or DNA) of the present invention which carries the DNA segments comprising the DNA vector which is to become part of the desired Product. The Vector Donor comprises a subcloning vector *D* (or it can be called the cloning vector if the Insert Donor does not already contain a cloning vector (e.g., for PCR fragments containing *attB* sites; see below)) and a segment *C* flanked by recombination sites (see Figure 1). Segments *C* and/or *D* can contain elements that contribute to selection for the desired Product daughter molecule, as described above for selection schemes. The recombination signals can be the same or different, and can be acted upon by the same or different recombinases. In addition, the Vector Donor can be linear or circular. Examples of such Vector Donor molecules include GATEWAY™ Destination Vectors, which include but are not limited to those Destination Vectors depicted in Figures 21-47 and 90-96.

**Primer:** refers to a single stranded or double stranded oligonucleotide that is extended by covalent bonding of nucleotide monomers during amplification or polymerization of a nucleic acid molecule (e.g. a DNA molecule). In a preferred aspect, a primer comprises one or more recombination sites or portions of such recombination sites. Portions of recombination sites comprise at least 2 bases (or basepairs, abbreviated herein as "bp"), at least 5-200 bases, at least 10-100 bases, at least 15-75 bases, at least 15-50 bases, at least 15-25 bases, or at least 16-25 bases, of the recombination sites of interest, as described in further detail below and in the Examples. When using portions of recombination sites, the missing portion of the recombination site may be provided as a template by the newly synthesized nucleic acid molecule. Such recombination sites may be located within and/or at one or both termini of the primer. Preferably, additional sequences are added to the primer adjacent to the recombination site(s) to enhance or improve recombination and/or to stabilize the recombination site during recombination. Such stabilization sequences may be any sequences (preferably G/C rich sequences) of any length. Preferably, such sequences range in size from 1 to about 1000 bases, 1 to about 500 bases, and 1 to about 100 bases, 1 to about 60 bases, 1 to about 25, 1 to about 10, 2 to about 10 and preferably about 4 bases.

Preferably, such sequences are greater than 1 base in length and preferably greater than 2 bases in length.

**Template:** refers to double stranded or single stranded nucleic acid molecules which are to be amplified, synthesized or sequenced. In the case of double stranded molecules, denaturation of its strands to form a first and a second strand is preferably performed before these molecules will be amplified, synthesized or sequenced, or the double stranded molecule may be used directly as a template. For single stranded templates, a primer complementary to a portion of the template is hybridized under appropriate conditions and one or more polypeptides having polymerase activity (e.g. DNA polymerases and/or reverse transcriptases) may then synthesize a nucleic acid molecule complementary to all or a portion of said template. Alternatively, for double stranded templates, one or more promoters may be used in combination with one or more polymerases to make nucleic acid molecules complementary to all or a portion of the template. The newly synthesized molecules, according to the invention, may be equal or shorter in length than the original template. Additionally, a population of nucleic acid templates may be used during synthesis or amplification to produce a population of nucleic acid molecules typically representative of the original template population.

**Adapter:** is an oligonucleotide or nucleic acid fragment or segment (preferably DNA) which comprises one or more recombination sites (or portions of such recombination sites) which in accordance with the invention can be added to a circular or linear Insert Donor molecule as well as other nucleic acid molecules described herein. When using portions of recombination sites, the missing portion may be provided by the Insert Donor molecule. Such adapters may be added at any location within a circular or linear molecule, although the adapters are preferably added at or near one or both termini of a linear molecule. Preferably, adapters are positioned to be located on both sides (flanking) a particular nucleic acid molecule of interest. In accordance with the invention, adapters may be added to nucleic acid molecules of interest by standard recombinant techniques (e.g. restriction digest and ligation). For example, adapters may be added to a circular molecule by first digesting the molecule with

an appropriate restriction enzyme, adding the adapter at the cleavage site and reforming the circular molecule which contains the adapter(s) at the site of cleavage. In other aspects, adapters may be added by homologous recombination, by integration of RNA molecules, and the like. Alternatively, adapters may be ligated directly to one or more and preferably both termini of a linear molecule thereby resulting in linear molecule(s) having adapters at one or both termini. In one aspect of the invention, adapters may be added to a population of linear molecules, (e.g. a cDNA library or genomic DNA which has been cleaved or digested) to form a population of linear molecules containing adapters at one and preferably both termini of all or substantial portion of said population.

**Adapter-Primer:** is primer molecule which comprises one or more recombination sites (or portions of such recombination sites) which in accordance with the invention can be added to a circular or linear nucleic acid molecule described herein. When using portions of recombination sites, the missing portion may be provided by a nucleic acid molecule (e.g., an adapter) of the invention. Such adapter-primers may be added at any location within a circular or linear molecule, although the adapter-primers are preferably added at or near one or both termini of a linear molecule. Examples of such adapter-primers and the use thereof in accordance with the methods of the invention are shown in Example 25 herein. Such adapter-primers may be used to add one or more recombination sites or portions thereof to circular or linear nucleic acid molecules in a variety of contexts and by a variety of techniques, including but not limited to amplification (e.g., PCR), ligation (e.g., enzymatic or chemical/synthetic ligation), recombination (e.g., homologous or non-homologous (illegitimate) recombination) and the like.

**Library:** refers to a collection of nucleic acid molecules (circular or linear). In one embodiment, a library may comprise a plurality (i.e., two or more) of DNA molecules, which may or may not be from a common source organism, organ, tissue, or cell. In another embodiment, a library is representative of all or a portion or a significant portion of the DNA content of an organism (a "genomic" library), or a set of nucleic acid molecules representative of all or a portion or a significant portion of the expressed nucleic acid molecules (a cDNA library) in a

cell, tissue, organ or organism. A library may also comprise random sequences made by *de novo* synthesis, mutagenesis of one or more sequences and the like. Such libraries may or may not be contained in one or more vectors.

**Amplification:** refers to any *in vitro* method for increasing a number of copies of a nucleotide sequence with the use of a polymerase. Nucleic acid amplification results in the incorporation of nucleotides into a DNA and/or RNA molecule or primer thereby forming a new molecule complementary to a template. The formed nucleic acid molecule and its template can be used as templates to synthesize additional nucleic acid molecules. As used herein, one amplification reaction may consist of many rounds of replication. DNA amplification reactions include, for example, polymerase chain reaction (PCR). One PCR reaction may consist of 5-100 "cycles" of denaturation and synthesis of a DNA molecule.

**Oligonucleotide:** refers to a synthetic or natural molecule comprising a covalently linked sequence of nucleotides which are joined by a phosphodiester bond between the 3' position of the deoxyribose or ribose of one nucleotide and the 5' position of the deoxyribose or ribose of the adjacent nucleotide. This term may be used interchangeably herein with the terms "nucleic acid molecule" and "polynucleotide," without any of these terms necessarily indicating any particular length of the nucleic acid molecule to which the term specifically refers.

**Nucleotide:** refers to a base-sugar-phosphate combination. Nucleotides are monomeric units of a nucleic acid molecule (DNA and RNA). The term nucleotide includes ribonucleoside triphosphates ATP, UTP, CTG, GTP and deoxyribonucleoside triphosphates such as dATP, dCTP, dITP, dUTP, dGTP, dTTP, or derivatives thereof. Such derivatives include, for example, [ $\alpha$ S]dATP, 7-deaza-dGTP and 7-deaza-dATP. The term nucleotide as used herein also refers to dideoxyribonucleoside triphosphates (ddNTPs) and their derivatives. Illustrated examples of dideoxyribonucleoside triphosphates include, but are not limited to, ddATP, ddCTP, ddGTP, ddITP, and ddTTP. According to the present invention, a "nucleotide" may be unlabeled or detectably labeled by well known techniques. Detectable labels include, for example, radioactive isotopes, fluorescent labels, chemiluminescent labels, bioluminescent labels and enzyme labels.



**Hybridization:** The terms “hybridization” and “hybridizing” refers to base pairing of two complementary single-stranded nucleic acid molecules (RNA and/or DNA) to give a double stranded molecule. As used herein, two nucleic acid molecules may be hybridized, although the base pairing is not completely complementary. Accordingly, mismatched bases do not prevent hybridization of two nucleic acid molecules provided that appropriate conditions, well known in the art, are used. In some aspects, hybridization is said to be under “stringent conditions.” By “stringent conditions” as used herein is meant overnight incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (150 mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt’s solution, 10% dextran sulfate, and 20 g/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C.

Other terms used in the fields of recombinant DNA technology and molecular and cell biology as used herein will be generally understood by one of ordinary skill in the applicable arts.

### *Overview*

Two reactions constitute the recombinational cloning system of the present invention, referred to herein as the “GATEWAY™ Cloning System,” as depicted generally in Figure 1. The first of these reactions, the **LR Reaction** (Figure 2), which may also be referred to interchangeably herein as the **Destination Reaction**, is the main pathway of this system. The LR Reaction is a recombination reaction between an Entry vector or clone and a Destination Vector, mediated by a cocktail of recombination proteins such as the GATEWAY™ LR Clonase™ Enzyme Mix described herein. This reaction transfers nucleic acid molecules of interest (which may be genes, cDNAs, cDNA libraries, or fragments thereof) from the Entry Clone to an Expression Vector, to create an Expression Clone.

The sites labeled L, R, B, and P are respectively the attL, attR, attB, and attP recombination sites for the bacteriophage  $\lambda$  recombination proteins that constitute the Clonase cocktail (referred to herein variously as “Clonase” or

“GATEWAY™ LR Clonase™ Enzyme Mix” (for recombination protein mixtures mediating attL x attR recombination reactions, as described herein) or “GATEWAY™ BP Clonase™ Enzyme Mix” (for recombination protein mixtures mediating attB x attP recombination reactions, as described herein)). The Recombinational Cloning reactions are equivalent to concerted, highly specific, cutting and ligation reactions. Viewed in this way, the recombination proteins cut to the left and right of the nucleic acid molecule of interest in the Entry Clone and ligate it into the Destination vector, creating a new Expression Clone.

The nucleic acid molecule of interest in an Expression Clone is flanked by the small attB1 and attB2 sites. The orientation and reading frame of the nucleic acid molecule of interest are maintained throughout the subcloning, because attL1 reacts only with attR1, and attL2 reacts only with attR2. Likewise, attB1 reacts only with attP1, and attB2 reacts only with attP2. Thus, the invention also relates to methods of controlled or directional cloning using the recombination sites of the invention (or portions thereof), including variants, fragments, mutants and derivatives thereof which may have altered or enhanced specificity. The invention also relates more generally to any number of recombination site partners or pairs (where each recombination site is specific for and interacts with its corresponding recombination site). Such recombination sites are preferably made by mutating or modifying the recombination site to provide any number of necessary specificities (e.g., attB1-10, attP1-10, attL1-10, attR1-10, etc.), non-limiting examples of which are described in detail in the Examples herein.

When an aliquot from the recombination reaction is transformed into host cells (e.g., *E. coli*) and spread on plates containing an appropriate selection agent, e.g., an antibiotic such as ampicillin with or without methicillin, cells that take up the desired clone form colonies. The unreacted Destination Vector does not give ampicillin-resistant colonies, even though it carries the ampicillin-resistance gene, because it contains a toxic gene, e.g., *ccdB*. Thus selection for ampicillin resistance selects for *E. coli* cells that carry the desired product, which usually comprise >90% of the colonies on the ampicillin plate.

To participate in the Recombinational (or “GATEWAY™”) Cloning Reaction, a nucleic acid molecule of interest first may be cloned into an Entry

Vector, creating an Entry Clone. Multiple options are available for creating Entry Clones, including: cloning of PCR sequences with terminal attB recombination sites into Entry Vectors; using the GATEWAY™ Cloning System recombination reaction; transfer of genes from libraries prepared in GATEWAY™ Cloning System vectors by recombination into Entry Vectors; and cloning of restriction enzyme-generated fragments and PCR fragments into Entry Vectors by standard recombinant DNA methods. These approaches are discussed in further detail herein.

A key advantage of the GATEWAY™ Cloning System is that a nucleic acid molecule of interest (or even a population of nucleic acid molecules of interest) present as an Entry Clone can be subcloned in parallel into one or more Destination Vectors in a simple reactions for anywhere from about 30 seconds to about 60 minutes (preferably about 1-60 minutes, about 1-45 minutes, about 1-30 minutes, about 2-60 minutes, about 2-45 minutes, about 2-30 minutes, about 1-2 minutes, about 30-60 minutes, about 45-60 minutes, or about 30-45 minutes). Longer reaction times (e.g., 2-24 hours, or overnight) may increase recombination efficiency, particularly where larger nucleic acid molecules are used, as described in the Examples herein. Moreover, a high percentage of the colonies obtained carry the desired Expression Clone. This process is illustrated schematically in Figure 3, which shows an advantage of the invention in which the molecule of interest can be moved simultaneously or separately into multiple Destination Vectors. In the LR Reaction, one or both of the nucleic acid molecules to be recombined may have any topology (e.g., linear, relaxed circular, nicked circular, supercoiled, etc.), although one or both are preferably linear.

The second major pathway of the GATEWAY™ Cloning System is the **BP Reaction** (Figure 4), which may also be referred to interchangeably herein as the **Entry Reaction** or the **Gateward Reaction**. The BP Reaction may recombine an Expression Clone with a Donor Plasmid (the counterpart of the byproduct in Figure 2). This reaction transfers the nucleic acid molecule of interest (which may have any of a variety of topologies, including linear, coiled, supercoiled, etc.) in the Expression Clone into an Entry Vector, to produce a new Entry Clone. Once this nucleic acid molecule of interest is cloned into an Entry

Vector, it can be transferred into new Expression Vectors, through the LR Reaction as described above. In the BP Reaction, one or both of the nucleic acid molecules to be recombined may have any topology (*e.g.*, linear, relaxed circular, nicked circular, supercoiled, etc.), although one or both are preferably linear.

5           A useful variation of the BP Reaction permits rapid cloning and expression of products of amplification (*e.g.*, PCR) or nucleic acid synthesis. Amplification (*e.g.*, PCR) products synthesized with primers containing terminal 25 bp attB sites serve as efficient substrates for the Gateway Cloning reaction. Such amplification products may be recombined with a Donor Vector to produce an Entry Clone (see 10   Figure 7). The result is an Entry Clone containing the amplification fragment. Such Entry Clones can then be recombined with Destination Vectors -- through the LR Reaction -- to yield Expression Clones of the PCR product.

          Additional details of the LR Reaction are shown in Figure 5A. The GATEWAY™ LR Clonase™ Enzyme Mix that mediates this reaction contains 15   lambda recombination proteins Int (Integrase), Xis (Excisionase), and IHF (Integration Host Factor). In contrast, the GATEWAY™ BP Clonase™ Enzyme Mix, which mediates the BP Reaction (Figure 5B), comprises Int and IHF alone.

          The recombination (att) sites of each vector comprise two distinct segments, donated by the parental vectors. The staggered lines dividing the two 20   portions of each att site, depicted in Figures 5A and 5B, represent the seven-base staggered cut produced by Int during the recombination reactions. This structure is seen in greater detail in Figure 6, which displays the attB recombination sequences of an Expression Clone, generated by recombination between the attL1 and attL2 sites of an Entry Clone and the attR1 and attR2 sites of a Destination 25   Vector.

          The nucleic acid molecule of interest in the Expression Clone is flanked by attB sites: attB1 to the left (amino terminus) and attB2 to the right (carboxy terminus). The bases in attB1 to the left of the seven-base staggered cut produced by Int are derived from the Destination vector, and the bases to the right of the 30   staggered cut are derived from the Entry Vector (see Figure 6). Note that the sequence is displayed in triplets corresponding to an open reading frame. If the reading frame of the nucleic acid molecule of interest cloned in the Entry Vector

is in phase with the reading frame shown for attB1, amino-terminal protein fusions can be made between the nucleic acid molecule of interest and any GATEWAY™ Cloning System Destination Vector encoding an amino-terminal fusion domain. Entry Vectors and Destination Vectors that enable cloning in all three reading frames are described in more detail herein, particularly in the Examples.

The LR Reaction allows the transfer of a desired nucleic acid molecule of interest into new Expression Vectors by recombining a Entry Clone with various Destination Vectors. To participate in the LR or Destination Reaction, however, a nucleic acid molecule of interest preferably is first converted to a Entry Clone. Entry Clones can be made in a number of ways, as shown in Figure 7.

One approach is to clone the nucleic acid molecule of interest into one or more of the Entry Vectors, using standard recombinant DNA methods, with restriction enzymes and ligase. The starting DNA fragment can be generated by restriction enzyme digestion or as a PCR product. The fragment is cloned between the attL1 and attL2 recombination sites in the Entry Vector. Note that a toxic or “death” gene (*e.g.*, *ccdB*), provided to minimize background colonies from incompletely digested Entry Vector, must be excised and replaced by the nucleic acid molecule of interest.

A second approach to making an Entry Clone (Figure 7) is to make a library (genomic or cDNA) in an Entry Vector, as described in detail herein. Such libraries may then be transferred into Destination Vectors for expression screening, for example in appropriate host cells such as yeast cells or mammalian cells.

A third approach to making Entry Clones (Figure 7) is to use Expression Clones obtained from cDNA molecules or libraries prepared in Expression Vectors. Such cDNAs or libraries, flanked by attB sites, can be introduced into a Entry Vector by recombination with a Donor Vector via the BP Reaction. If desired, an entire Expression Clone library can be transferred into the Entry Vector through the BP Reaction. Expression Clone cDNA libraries may also be constructed in a variety of prokaryotic and eukaryotic GATEWAY™-modified vectors (*e.g.*, the pEXP501 Expression Vector (see Figure 48), and 2-hybrid and

attB library vectors), as described in detail herein, particularly in the Examples below.

A fourth, and potentially most versatile, approach to making an Entry Clone (Figure 7) is to introduce a sequence for a nucleic acid molecule of interest into an Entry Vector by amplification (*e.g.*, PCR) fragment cloning. This method is diagramed in Figure 8. The DNA sequence first is amplified (for example, with PCR) as outlined in detail below and in the Examples herein, using primers containing one or more bp, two or more bp, three or more bp, four or more bp, five or more bp, preferably six or more bp, more preferably 6-25 bp (particularly 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25) bp of the attB nucleotide sequences (such as, but not limited to, those depicted in Figure 9), and optionally one or more, two or more, three or more, four or more, and most preferably four or five or more additional terminal nucleotide bases which preferably are guanines. The PCR product then may be converted to a Entry Clone by performing a BP Reaction, in which the attB-PCR product recombines with a Donor Vector containing one or more attP sites. Details of this approach and protocols for PCR fragment subcloning are provided in Examples 8 and 21-25.

A variety of Entry Clones may be produced by these methods, providing a wide array of cloning options; a number of specific Entry Vectors are also available commercially from Life Technologies, Inc. (Rockville, MD). The Examples herein provide a more in-depth description of selected Entry Vectors and details of their cloning sites. Choosing the optimal Entry Vector for a particular application is discussed in Example 4.

Entry Vectors and Destination Vectors should be constructed so that the amino-terminal region of a nucleic acid molecule of interest (*e.g.*, a gene, cDNA library or insert, or fragment thereof) will be positioned next to the attL1 site. Entry Vectors preferably contain the *rrnB* transcriptional terminator upstream of the attL1 site. This sequence ensures that expression of cloned nucleic acid molecules of interest is reliably "off" in *E. coli*, so that even toxic genes can be successfully cloned. Thus, Entry Clones may be designed to be transcriptionally silent. Note also that Entry Vectors, and hence Entry Clones, may contain the kanamycin antibiotic resistance (*kan<sup>r</sup>*) gene to facilitate selection of host cells

containing Entry Clones after transformation. In certain applications, however, Entry Clones may contain other selection markers, including but not limited to a gentamycin resistance (*gen<sup>r</sup>*) or tetracycline resistance (*tet<sup>r</sup>*) gene, to facilitate selection of host cells containing Entry Clones after transformation.

5           Once a nucleic acid molecule of interest has been cloned into an Entry Vector, it may be moved into a Destination Vector. The upper right portion of Figure 5A shows a schematic of a Destination Vector. The thick arrow represents some function (often transcription or translation) that will act on the nucleic acid molecule of interest in the clone. During the recombination reaction, the region  
10           between the attR1 and attR2 sites, including a toxic or "death" gene (*e.g.*, *ccdB*), is replaced by the DNA segment from the Entry Clone. Selection for recombinants that have acquired the ampicillin resistance (*amp<sup>r</sup>*) gene (carried on the Destination Vector) and that have also lost the death gene ensures that a high percentage (usually >90%) of the resulting colonies will contain the correct insert.

15           To move a nucleic acid molecule of interest into a Destination Vector, the Destination Vector is mixed with the Entry Clone comprising the desired nucleic acid molecule of interest, a cocktail of recombination proteins (*e.g.*, GATEWAY™ LR Clonase™ Enzyme Mix) is added, the mixture is incubated (preferably at about 25°C for about 60 minutes, or longer under certain  
20           circumstances, *e.g.* for transfer of large nucleic acid molecules, as described below) and any standard host cell (including bacterial cells such as *E. coli*; animal cells such as insect cells, mammalian cells, nematode cells and the like; plant cells; and yeast cells) strain is transformed with the reaction mixture. The host cell used will be determined by the desired selection (*e.g.*, *E. coli* DB3.1, available  
25           commercially from Life Technologies, Inc., allows survival of clones containing the *ccdB* death gene, and thus can be used to select for cointegrate molecules -- *i.e.*, molecules that are hybrids between the Entry Clone and Destination Vector). The Examples below provide further details and protocols for use of Entry and Destination Vectors in transferring nucleic acid molecules of interest and  
30           expressing RNAs or polypeptides encoded by these nucleic acid molecules in a variety of host cells.

The cloning system of the invention therefore offers multiple advantages:

- Once a nucleic acid molecule of interest is cloned into the GATEWAY™ Cloning System, it can be moved into and out of other vectors with complete fidelity of reading frame and orientation. That is, since the reactions proceed whereby attL1 on the Entry Clone recombines with attR1 on the Destination Vector, the directionality of the nucleic acid molecule of interest is maintained or may be controlled upon transfer from the Entry Clone into the Destination Vector. Hence, the GATEWAY™ Cloning System provides a powerful and easy method of directional cloning of nucleic acid molecule of interest.
- One-step cloning or subcloning: Mix the Entry Clone and the Destination Vector with Clonase, incubate, and transform.
- Clone PCR products readily by *in vitro* recombination, by adding attB sites to PCR primers. Then directly transfer these Entry Clones into Destination Vectors. This process may also be carried out in one step (see Examples below).
- Powerful selections give high reliability: >90% ( and often >99%) of the colonies contain the desired DNA in its new vector.
- One-step conversion of existing standard vectors into GATEWAY™ Cloning System vectors.
- Ideal for large vectors or those with few cloning sites.
- Recombination sites are short (25 bp), and may be engineered to contain no stop codons or secondary structures.
- Reactions may be automated, for high-throughput applications (*e.g.*, for diagnostic purposes or for therapeutic candidate screening).
- The reactions are economical: 0.3 µg of each DNA; no restriction enzymes, phosphatase, ligase, or gel purification. Reactions work well with miniprep DNA.
- Transfer multiple clones, and even libraries, into one or more Destination Vectors, in a single experiment.
- A variety of Destination Vectors may be produced, for applications including, but not limited to:



- Protein expression in *E. coli*: native proteins; fusion proteins with GST, His6, thioredoxin, etc., for purification, or one or more epitope tags; any promoter useful in expressing proteins in *E. coli* may be used, such as ptrc,  $\lambda P_L$ , and T7 promoters.
- Protein expression in eukaryotic cells: CMV promoter, baculovirus (with or without His6 tag), Semliki Forest virus, Tet regulation.
- DNA sequencing (all *lac* primers), RNA probes, phagemids (both strands)
- A variety of Entry Vectors (for recombinational cloning entry by standard recombinant DNA methods) may be produced:
  - Strong transcription stop just upstream, for genes toxic to *E. coli*.
  - Three reading frames.
  - With or without TEV protease cleavage site.
  - Motifs for prokaryotic and / or eukaryotic translation.
  - Compatible with commercial cDNA libraries.
- Expression Clone cDNA (attB) libraries, for expression screening, including 2-hybrid libraries and phage display libraries, may also be constructed.

### ***Recombination Site Sequences***

In one aspect, the invention relates to nucleic acid molecules, which may or may not be isolated nucleic acid molecules, comprising one or more nucleotide sequences encoding one or more recombination sites or portions thereof. In particular, this aspect of the invention relates to such nucleic acid molecules comprising one or more nucleotide sequences encoding *attB*, *attP*, *attL*, or *attR*, or portions of these recombination site sequences. The invention also relates to mutants, derivatives, and fragments of such nucleic acid molecules. Unless otherwise indicated, all nucleotide sequences that may have been determined by sequencing a DNA molecule herein were determined using manual or automated DNA sequencing, such as dideoxy sequencing, according to methods that are routine to one of ordinary skill in the art (Sanger, F., and Coulson, A.R., *J. Mol. Biol.* 94:444-448 (1975); Sanger, F., *et al.*, *Proc. Natl. Acad. Sci. USA* 74:5463-5467 (1977)). All amino acid sequences of polypeptides encoded by DNA

molecules determined herein were predicted by conceptual translation of a DNA sequence determined as above. Therefore, as is known in the art for any DNA sequence determined by these approaches, any nucleotide sequence determined herein may contain some errors. Nucleotide sequences determined by such methods are typically at least about 90% identical, more typically at least about 95% to at least about 99.9% identical to the actual nucleotide sequence of the sequenced DNA molecule. As is also known in the art, a single insertion or deletion in a determined nucleotide sequence compared to the actual sequence will cause a frame shift in translation of the nucleotide sequence such that the predicted amino acid sequence encoded by a determined nucleotide sequence will be completely different from the amino acid sequence actually encoded by the sequenced DNA molecule, beginning at the point of such an insertion or deletion.

Unless otherwise indicated, each "nucleotide sequence" set forth herein is presented as a sequence of deoxyribonucleotides (abbreviated A, G, C and T). However, by "nucleotide sequence" of a nucleic acid molecule or polynucleotide is intended, for a DNA molecule or polynucleotide, a sequence of deoxyribonucleotides, and for an RNA molecule or polynucleotide, the corresponding sequence of ribonucleotides (A, G, C and U), where each thymidine deoxyribonucleotide (T) in the specified deoxyribonucleotide sequence is replaced by the ribonucleotide uridine (U). Thus, the invention relates to sequences of the invention in the form of DNA or RNA molecules, or hybrid DNA/RNA molecules, and their corresponding complementary DNA, RNA, or DNA/RNA strands.

In a first such aspect, the invention provides nucleic acid molecules comprising one or more nucleotide sequences encoding *attB1*, or mutants, fragments, variants or derivatives thereof. Such nucleic acid molecules may comprise an *attB1* nucleotide sequence having the sequence set forth in Figure 9, such as: ACAAGTTTGTACAAAAAAGCAGGCT, or a nucleotide sequence complementary to the nucleotide sequence set forth in Figure 9 for *attB1*, or mutants, fragments, variants or derivatives thereof. As one of ordinary skill will appreciate, however, certain mutations, insertions, or deletions of one or more bases in the *attB1* sequence contained in the nucleic acid molecules of the invention may be made without compromising the structural and functional

integrity of these molecules; hence, nucleic acid molecules comprising such mutations, insertions, or deletions in the *attB1* sequence are encompassed within the scope of the invention.

In a related aspect, the invention provides nucleic acid molecules comprising one or more nucleotide sequences encoding *attB2*, or mutants, fragments, variants or derivatives thereof. Such nucleic acid molecules may comprise an *attB2* nucleotide sequence having the sequence set forth in Figure 9, such as: ACCCAGCTTTCTTGTACAAAGTGGT, or a nucleotide sequence complementary to the nucleotide sequence set forth in Figure 9 for *attB2*, or mutants, fragments, variants or derivatives thereof. As noted above for *attB1*, certain mutations, insertions, or deletions of one or more bases in the *attB2* sequence contained in the nucleic acid molecules of the invention may be made without compromising the structural and functional integrity of these molecules; hence, nucleic acid molecules comprising such mutations, insertions, or deletions in the *attB2* sequence are encompassed within the scope of the invention.

A recombinant host cell comprising a nucleic acid molecule containing *attB1* and *attB2* sites (the vector pEXP501, also known as pCMVSPORT6; see Figure 48), *E. coli* DB3.1(pCMVSPORT6), was deposited on February 27, 1999, with the Collection, Agricultural Research Culture Collection (NRRL), 1815 North University Street, Peoria, Illinois 61604 USA, as Deposit No. NRRL B-30108. The *attB1* and *attB2* sites within the deposited nucleic acid molecule are contained in nucleic acid cassettes in association with one or more additional functional sequences as described in more detail below.

In another related aspect, the invention provides nucleic acid molecules comprising one or more nucleotide sequences encoding *attP1*, or mutants, fragments, variants or derivatives thereof. Such nucleic acid molecules may comprise an *attP1* nucleotide sequence having the sequence set forth in Figure 9, such as: TACAGGTCACCTAATACCATCTAAGTAGTTGATTCATAGTGA-CTGGATATGTTGTGTTTTACAGTATTATGTAGTCTGTTTTTTAT-GCAAAATCTAATTTAATATATTGATATTTATATCATTTTACGTT-TCTCGTTCAGCTTTTTTGTACAAAGTTGGCATTATAAAAAAGCATTG-CTCATCAATTTGTTGCAACGAACAGGTCATCATCAGTCAAAATAA-

AATCATTATTG, or a nucleotide sequence complementary to the nucleotide sequence set forth in Figure 9 for *attP1*, or mutants, fragments, variants or derivatives thereof. As noted above for *attB1*, certain mutations, insertions, or deletions of one or more bases in the *attP1* sequence contained in the nucleic acid molecules of the invention may be made without compromising the structural and functional integrity of these molecules; hence, nucleic acid molecules comprising such mutations, insertions, or deletions in the *attP1* sequence are encompassed within the scope of the invention.

In another related aspect, the invention provides nucleic acid molecules comprising one or more nucleotide sequences encoding *attP2*, or mutants, fragments, variants or derivatives thereof. Such nucleic acid molecules may comprise an *attP2* nucleotide sequence having the sequence set forth in Figure 9, such as: CAAATAATGATTTTATTTGACTGATAGTGACCTGTTCGTTG-CAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGCTGAACGAGAAACGTAAAATGATA-TAAATATCAATATATTAAATTAGATTTTGCATAAAAAACAG-ACTACATAATACTGTAAAACACAACATATCCAGTCACTATGAATCAACTACTTAGATGGTATTAGTGACCTGTA, or a nucleotide sequence complementary to the nucleotide sequence set forth in Figure 9 for *attP2*, or mutants, fragments, variants or derivatives thereof. As noted above for *attB1*, certain mutations, insertions, or deletions of one or more bases in the *attP2* sequence contained in the nucleic acid molecules of the invention may be made without compromising the structural and functional integrity of these molecules; hence, nucleic acid molecules comprising such mutations, insertions, or deletions in the *attP2* sequence are encompassed within the scope of the invention.

A recombinant host cell comprising a nucleic acid molecule (the attP vector pDONR201, also known as pENTR21-attPkan or pAttPkan; see Figure 49) containing attP1 and attP2 sites, *E. coli* DB3.1(pAttPkan) (also called *E. coli* DB3.1(pAHKAn)), was deposited on February 27, 1999, with the Collection, Agricultural Research Culture Collection (NRRL), 1815 North University Street, Peoria, Illinois 61604 USA, as Deposit No. NRRL B-30099. The attP1 and attP2 sites within the deposited nucleic acid molecule are contained in nucleic acid

cassettes in association with one or more additional functional sequences as described in more detail below.

In another related aspect, the invention provides nucleic acid molecules comprising one or more nucleotide sequences encoding *attR1*, or mutants, fragments, variants or derivatives thereof. Such nucleic acid molecules may comprise an *attR1* nucleotide sequence having the sequence set forth in Figure 9, such as: ACAAGTTTGTACAAAAAGCTGAACGAG-AAACGTAAATGATATAAATATCAATATATTAAATTAGATTTTGCAT-AAAAACAGACTACATAATACTGTAAAACACAACATATCCAGTCA-CTATG, or a nucleotide sequence complementary to the nucleotide sequence set forth in Figure 9 for *attR1*, or mutants, fragments, variants or derivatives thereof. As noted above for *attB1*, certain mutations, insertions, or deletions of one or more bases in the *attR1* sequence contained in the nucleic acid molecules of the invention may be made without compromising the structural and functional integrity of these molecules; hence, nucleic acid molecules comprising such mutations, insertions, or deletions in the *attR1* sequence are encompassed within the scope of the invention.

In another related aspect, the invention provides nucleic acid molecules comprising one or more nucleotide sequences encoding *attR2*, or mutants, fragments, variants or derivatives thereof. Such nucleic acid molecules may comprise an *attR2* nucleotide sequence having the sequence set forth in Figure 9, such as: GCAGGTCGACCATAGTGACTGGATAT-GTTGTGTTTTACAGTATTATGTAGTCTGTTTTTTATGCAAAATCTA-ATTTAATATATTGATATTTATATCATTTTACGTTTCTCGTTCAGCTT-TCTTGTACAAAGTGGT, or a nucleotide sequence complementary to the nucleotide sequence set forth in Figure 9 for *attR2*, or mutants, fragments, variants or derivatives thereof. As noted above for *attB1*, certain mutations, insertions, or deletions of one or more bases in the *attR2* sequence contained in the nucleic acid molecules of the invention may be made without compromising the structural and functional integrity of these molecules; hence, nucleic acid molecules comprising such mutations, insertions, or deletions in the *attR2* sequence are encompassed within the scope of the invention.

Recombinant host cell strains containing attR1 sites apposed to cloning sites in reading frame A, reading frame B, and reading frame C, *E. coli* DB3.1(pEZC15101) (reading frame A; see Figure 64A), *E. coli* DB3.1(pEZC15102) (reading frame B; see Figure 64B), and *E. coli* DB3.1(pEZC15103) (reading frame C; see Figure 64C), and containing corresponding attR2 sites, were deposited on February 27, 1999, with the Collection, Agricultural Research Culture Collection (NRRL), 1815 North University Street, Peoria, Illinois 61604 USA, as Deposit Nos. NRRL B-30103, NRRL B-30104, and NRRL B-30105, respectively. The attR1 and attR2 sites within the deposited nucleic acid molecules are contained in nucleic acid cassettes in association with one or more additional functional sequences as described in more detail below.

In another related aspect, the invention provides nucleic acid molecules comprising one or more nucleotide sequences encoding attL1, or mutants, fragments, variants and derivatives thereof. Such nucleic acid molecules may comprise an attL1 nucleotide sequence having the sequence set forth in Figure 9, such as: CAA ATA ATG ATT TTA TTT TGA CTG ATA GTG ACC TGT TCG TTG CAA CAA ATT GAT AAG CAA TGC TTT TTT ATA ATG CCA ACT TTG TAC AAA AAA GCA GGC T, or a nucleotide sequence complementary to the nucleotide sequence set forth in Figure 9 for attL1, or mutants, fragments, variants or derivatives thereof. As noted above for attB1, certain mutations, insertions, or deletions of one or more bases in the attL1 sequence contained in the nucleic acid molecules of the invention may be made without compromising the structural and functional integrity of these molecules; hence, nucleic acid molecules comprising such mutations, insertions, or deletions in the attL1 sequence are encompassed within the scope of the invention.

In another related aspect, the invention provides nucleic acid molecules comprising one or more nucleotide sequences encoding attL2, or mutants, fragments, variants and derivatives thereof. Such nucleic acid molecules may comprise an attL2 nucleotide sequence having the sequence set forth in Figure 9, such as: C AAA TAA TGA TTT TAT TTT GAC TGA TAG TGA CCT GTT CGT TGC AAC AAA TTG ATA AGC AAT GCT TTC TTA TAA TGC CAA

CTT TGT ACA AGA AAG CTG GGT, or a nucleotide sequence complementary to the nucleotide sequence set forth in Figure 9 for *attL2*, or mutants, fragments, variants or derivatives thereof. As noted above for *attB1*, certain mutations, insertions, or deletions of one or more bases in the *attL2* sequence contained in the nucleic acid molecules of the invention may be made without compromising the structural and functional integrity of these molecules; hence, nucleic acid molecules comprising such mutations, insertions, or deletions in the *attL2* sequence are encompassed within the scope of the invention.

Recombinant host cell strains containing *attL1* sites apposed to cloning sites in reading frame A, reading frame B, and reading frame C, *E. coli* DB3.1(pENTR1A) (reading frame A; see Figure 10), *E. coli* DB3.1(pENTR2B) (reading frame B; see Figure 11), and *E. coli* DB3.1(pENTR3C) (reading frame C; see Figure 12), and containing corresponding *attL2* sites, were deposited on February 27, 1999, with the Collection, Agricultural Research Culture Collection (NRRL), 1815 North University Street, Peoria, Illinois 61604 USA, as Deposit Nos. NRRL B-30100, NRRL B-30101, and NRRL B-30102, respectively. The *attL1* and *attL2* sites within the deposited nucleic acid molecules are contained in nucleic acid cassettes in association with one or more additional functional sequences as described in more detail below.

Each of the recombination site sequences described herein or portions thereof, or the nucleotide sequence cassettes contained in the deposited clones, may be cloned or inserted into a vector of interest (for example, using the recombinational cloning methods described herein and/or standard restriction cloning techniques that are routine in the art) to generate, for example, Entry Vectors or Destination Vectors which may be used to transfer a desired segment of a nucleic acid molecule of interest (*e.g.*, a gene, cDNA molecule, or cDNA library) into a desired vector or into a host cell.

Using the information provided herein, such as the nucleotide sequences for the recombination site sequences described herein, an isolated nucleic acid molecule of the present invention encoding one or more recombination sites or portions thereof may be obtained using standard cloning and screening procedures, such as those for cloning cDNAs using mRNA as starting material. Preferred such

methods include PCR-based cloning methods, such as reverse transcriptase-PCR (RT-PCR) using primers such as those described herein and in the Examples below. Alternatively, vectors comprising the cassettes containing the recombination site sequences described herein are available commercially from Life Technologies, Inc. (Rockville, MD).

The invention is also directed to nucleic acid molecules comprising one or more of the recombination site sequences or portions thereof and one or more additional nucleotide sequences, which may encode functional or structural sites such as one or more multiple cloning sites, one or more transcription termination sites, one or more transcriptional regulatory sequences (which may be promoters, enhancers, repressors, and the like), one or more translational signals (*e.g.*, secretion signal sequences), one or more origins of replication, one or more fusion partner peptides (particularly glutathione S-transferase (GST), hexahistidine (His<sub>6</sub>), and thioredoxin (Trx)), one or more selection markers or modules, one or more nucleotide sequences encoding localization signals such as nuclear localization signals or secretion signals, one or more origins of replication, one or more protease cleavage sites, one or more genes or portions of genes encoding a protein or polypeptide of interest, and one or more 5' polynucleotide extensions (particularly an extension of guanine residues ranging in length from about 1 to about 20, from about 2 to about 15, from about 3 to about 10, from about 4 to about 10, and most preferably an extension of 4 or 5 guanine residues at the 5' end of the recombination site nucleotide sequence. The one or more additional functional or structural sequences may or may not flank one or more of the recombination site sequences contained on the nucleic acid molecules of the invention.

In some nucleic acid molecules of the invention, the one or more nucleotide sequences encoding one or more additional functional or structural sites may be operably linked to the nucleotide sequence encoding the recombination site. For example, certain nucleic acid molecules of the invention may have a promoter sequence operably linked to a nucleotide sequence encoding a recombination site or portion thereof of the invention, such as a T7 promoter, a phage lambda PL



promoter, an *E. coli lac*, *trp* or *tac* promoter, and other suitable promoters which will be familiar to the skilled artisan.

Nucleic acid molecules of the present invention, which may be isolated nucleic acid molecules, may be in the form of RNA, such as mRNA, or in the form of DNA, including, for instance, cDNA and genomic DNA obtained by cloning or produced synthetically, or in the form of DNA-RNA hybrids. The nucleic acid molecules of the invention may be double-stranded or single-stranded. Single-stranded DNA or RNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand. The nucleic acid molecules of the invention may also have a number of topologies, including linear, circular, coiled, or supercoiled.

By "isolated" nucleic acid molecule(s) is intended a nucleic acid molecule, DNA or RNA, which has been removed from its native environment. For example, recombinant DNA molecules contained in a vector are considered isolated for the purposes of the present invention. Further examples of isolated DNA molecules include recombinant DNA molecules maintained in heterologous host cells, and those DNA molecules purified (partially or substantially) from a solution whether produced by recombinant DNA or synthetic chemistry techniques. Isolated RNA molecules include *in vivo* or *in vitro* RNA transcripts of the DNA molecules of the present invention.

The present invention further relates to mutants, fragments, variants and derivatives of the nucleic acid molecules of the present invention, which encode portions, analogs or derivatives of one or more recombination sites. Variants may occur naturally, such as a natural allelic variant. By an "allelic variant" is intended one of several alternate forms of a gene occupying a given locus on a chromosome of an organism (*see* Lewin, B., ed., *Genes II*, , John Wiley & Sons, New York (1985)). Non-naturally occurring variants may be produced using art-known mutagenesis techniques, such as those described hereinbelow.

Such variants include those produced by nucleotide substitutions, deletions or additions or portions thereof, or combinations thereof. The substitutions, deletions or additions may involve one or more nucleotides. The variants may be altered in coding regions, non-coding regions, or both. Alterations in the coding

regions may produce conservative or non-conservative amino acid substitutions, deletions or additions. Especially preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of the encoded polypeptide(s) or portions thereof, and which also do not substantially alter the reactivities of the recombination site nucleic acid sequences in recombination reactions. Also especially preferred in this regard are conservative substitutions.

Particularly preferred mutants, fragments, variants, and derivatives of the nucleic acid molecules of the invention include, but are not limited to, insertions, deletions or substitutions of one or more nucleotide bases within the 15 bp core region (GCTTTTTTATACTAA) which is identical in all four wildtype lambda *att* sites, *attB*, *attP*, *attL* and *attR* (see U.S. Application Nos. 08/663,002, filed June 7, 1996 (now U.S. Patent No. 5,888,732), 09/005,476, filed January 12, 1998, and 09/177,387, filed October 23, 1998, which describes the core region in further detail, and the disclosures of which are incorporated herein by reference in their entireties). Analogously, the core regions in *attB*1, *attP*1, *attL*1 and *attR*1 are identical to one another, as are the core regions in *attB*2, *attP*2, *attL*2 and *attR*2. Particularly preferred in this regard are nucleic acid molecules comprising insertions, deletions or substitutions of one or more nucleotides within the seven bp overlap region (TTTATAC, which is defined by the cut sites for the integrase protein and is the region where strand exchange takes place) that occurs within this 15 bp core region (GCTTTTTTATACTAA). Examples of such preferred mutants, fragments, variants and derivatives according to this aspect of the invention include, but are not limited to, nucleic acid molecules in which the thymine at position 1 of the seven bp overlap region has been deleted or substituted with a guanine, cytosine, or adenine; in which the thymine at position 2 of the seven bp overlap region has been deleted or substituted with a guanine, cytosine, or adenine; in which the thymine at position 3 of the seven bp overlap region has been deleted or substituted with a guanine, cytosine, or adenine; in which the adenine at position 4 of the seven bp overlap region has been deleted or substituted with a guanine, cytosine, or thymine; in which the thymine at position 5 of the seven bp overlap region has been deleted or substituted with a

guanine, cytosine, or adenine; in which the adenine at position 6 of the seven bp overlap region has been deleted or substituted with a guanine, cytosine, or thymine; and in which the cytosine at position 7 of the seven bp overlap region has been deleted or substituted with a guanine, thymine, or adenine; or any combination of one or more such deletions and/or substitutions within this seven bp overlap region. As described in detail in Example 21 herein, mutants of the nucleic acid molecules of the invention in which substitutions have been made within the first three positions of the seven bp overlap (TTTATAC) have been found in the present invention to strongly affect the specificity of recombination, mutant nucleic acid molecules in which substitutions have been made in the last four positions (TTTATAC) only partially alter recombination specificity, and mutant nucleic acid molecules comprising nucleotide substitutions outside of the seven bp overlap, but elsewhere within the 15 bp core region, do not affect specificity of recombination but do influence the efficiency of recombination.

Hence, in an additional aspect, the present invention is also directed to nucleic acid molecules comprising one or more recombination site nucleotide sequences that affect recombination specificity, particularly one or more nucleotide sequences that may correspond substantially to the seven base pair overlap within the 15 bp core region, having one or more mutations that affect recombination specificity. Particularly preferred such molecules may comprise a consensus sequence (described in detail in Example 21 herein) such as NNNATAC, wherein "N" refers to any nucleotide (*i.e.*, may be A, G, T/U or C), with the proviso that if one of the first three nucleotides in the consensus sequence is a T/U, then at least one of the other two of the first three nucleotides is not a T/U.

In a related aspect, the present invention is also directed to nucleic acid molecules comprising one or more recombination site nucleotide sequences that enhance recombination efficiency, particularly one or more nucleotide sequences that may correspond substantially to the core region and having one or more mutations that enhance recombination efficiency. By sequences or mutations that "enhance recombination efficiency" is meant a sequence or mutation in a recombination site, preferably in the core region (*e.g.*, the 15 bp core region of *att* recombination sites), that results in an increase in cloning efficiency (typically

measured by determining successful cloning of a test sequence, *e.g.*, by determining CFU/ml for a given cloning mixture) when recombining molecules comprising the mutated sequence or core region as compared to molecules that do not comprise the mutated sequence or core region (*e.g.*, those comprising a wildtype recombination site core region sequence). More specifically, whether or not a given sequence or mutation enhances recombination efficiency may be determined using the sequence or mutation in recombinational cloning as described herein, and determining whether the sequence or mutation provides enhanced recombinational cloning efficiency when compared to a non-mutated (*e.g.*, wildtype) sequence. Methods of determining preferred cloning efficiency-enhancing mutations for a number of recombination sites, particularly for *att* recombination sites, are described herein, for example in Examples 22-25. Examples of preferred such mutant recombination sites include but are not limited to the *attL* consensus core sequence of caactntntnnnannaagttg (wherein "n" represents any nucleotide), for example the *attL5* sequence agcctgctttattataactaagttggcatta and the *attL6* sequence agcctgcttttttatattaagttggcatta; the *attB1.6* sequence ggggacaactttgtacaaaaaagttggct; the *attB2.2* sequence ggggacaactttgtacaagaaagctgggt; and the *attB2.10* sequence ggggacaactttgtacaagaaagttgggt. Those of skill in the art will appreciate that, in addition to the core region, other portions of the *att* site may affect the efficiency of recombination. There are five so-called arm binding sites for the integrase protein in the bacteriophage lambda *attP* site, two in *attR* (P1 and P2), and three in *attL* (P'1, P'2 and P'3). Compared to the core binding sites, the integrase protein binds to arm sites with high affinity and interacts with core and arm sites through two different domains of the protein. As with the core binding site a consensus sequence for the arm binding site consisting of C/AAGTCACTAT has been inferred from sequence comparison of the five arm binding sites and seven non-*att* sites (Ross and Landy, *Proc. Natl. Acad. Sci. USA* 79:7724-7728 (1982)). Each arm site has been mutated and tested for its effect in the excision and integration reactions (Numrych *et al.*, *Nucl. Acids Res.* 18:3953 (1990)). Hence, specific sites are utilized in each reaction in different ways, namely, the P1 and P'3

sites are essential for the integration reaction whereas the other three sites are dispensable to the integration reaction to varying degrees. Similarly, the P2, P'1 and P'2 sites are most important for the excision reaction, whereas P1 and P'3 are completely dispensable. Interestingly, when P2 is mutated the integration reaction occurs more efficiently than with the wild type attP site. Similarly, when P1 and P'3 are mutated the excision reaction occurs more efficiently. The stimulatory effect of mutating integrase arm binding sites can be explained by removing sites that compete or inhibit a specific recombination pathway or that function in a reaction that converts products back to starting substrates. In fact there is evidence for an XIS-independent LR reaction (Abremski and Gottesman, *J. Mol. Biol.* 153:67-78 (1981)). Thus, in addition to modifications in the core region of the att site, the present invention contemplates the use of att sites containing one or more modifications in the integrase arm-type binding sites. In some preferred embodiments, one or more mutations may be introduced into one or more of the P1, P'1, P2, P'2 and P'3 sites. In some preferred embodiments, multiple mutations may be introduced into one or more of these sites. Preferred such mutations include those which increase the recombination *in vitro*. For example, in some embodiments mutations may be introduced into the arm-type binding sites such that integrative recombination, corresponding to the BP reaction, is enhanced. In other embodiments, mutations may be introduced into the arm-type binding sites such that excisive recombination, corresponding to the LR reaction, is enhanced. Of course, based on the guidance contained herein, particularly in the construction and evaluation of effects of mutated recombination sites upon recombinational specificity and efficiency, analogous mutated or engineered sequences may be produced for other recombination sites described herein (including but not limited to *lox*, FRT, and the like) and used in accordance with the invention. For example, much like the mutagenesis strategy used to select core binding sites that enhance recombination efficiency, similar strategies can be employed to select changes in the arms of attP, attL and attR, and in analogous sequences in other recombination sites such as *lox*, FRT and the like, that enhance recombination efficiency. Hence, the construction and evaluation of such mutants is well within the abilities of those of ordinary skill in the art without undue experimentation.

One suitable methodology for preparing and evaluating such mutations is found in Numrych, *et al.*, (1990) *Nucleic Acids Research* 18(13): 3953-3959.

Other mutant sequences and nucleic acid molecules that may be suitable to enhance recombination efficiency will be apparent from the description herein, or may be easily determined by one of ordinary skill using only routine experimentation in molecular biology in view of the description herein and information that is readily available in the art

Since the genetic code is well known in the art, it is also routine for one of ordinary skill in the art to produce degenerate variants of the nucleic acid molecules described herein without undue experimentation. Hence, nucleic acid molecules comprising degenerate variants of nucleic acid sequences encoding the recombination sites described herein are also encompassed within the scope of the invention.

Further embodiments of the invention include isolated nucleic acid molecules comprising a polynucleotide having a nucleotide sequence at least 50% identical, at least 60% identical, at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, and more preferably at least 95%, 96%, 97%, 98% or 99% identical to the nucleotide sequences of the seven bp overlap region within the 15 bp core region of the recombination sites described herein, or the nucleotide sequences of *attB1*, *attB2*, *attP1*, *attP2*, *attL1*, *attL2*, *attR1* or *attR2* as set forth in Figure 9 (or portions thereof), or a nucleotide sequence complementary to any of these nucleotide sequences, or fragments, variants, mutants, and derivatives thereof.

By a polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence encoding a particular recombination site or portion thereof is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations (*e.g.*, insertions, substitutions, or deletions) per each 100 nucleotides of the reference nucleotide sequence encoding the recombination site. For example, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference *attB1* nucleotide sequence, up to 5% of the nucleotides in the *attB1* reference sequence may be

deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the *attB1* reference sequence may be inserted into the *attB1* reference sequence. These mutations of the reference sequence may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or  
5 anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular nucleic acid molecule is at least 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical  
10 to, for instance, a given recombination site nucleotide sequence or portion thereof can be determined conventionally using known computer programs such as DNAsis software (Hitachi Software, San Bruno, California) for initial sequence alignment followed by ESEE version 3.0 DNA/protein sequence software (cabot@trog.mbb.sfu.ca) for multiple sequence alignments. Alternatively, such  
15 determinations may be accomplished using the BESTFIT program (Wisconsin Sequence Analysis Package, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711), which employs a local homology algorithm (Smith and Waterman, *Advances in Applied Mathematics* 2: 482-489 (1981)) to find the best segment of homology between two sequences. When  
20 using DNAsis, ESEE, BESTFIT or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set such that the percentage of identity is calculated over the full length of the reference nucleotide sequence and that gaps in homology of up to 5% of the total number  
25 of nucleotides in the reference sequence are allowed.

The present invention is directed to nucleic acid molecules at least 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the *attB1*, *attB2*, *attP1*, *attP2*, *attL1*, *attL2*, *attR1* or *attR2* nucleotide sequences as set forth in Figure 9, or to the nucleotide sequence of the deposited clones, irrespective of  
30 whether they encode particular functional polypeptides. This is because even where a particular nucleic acid molecule does not encode a particular functional polypeptide, one of skill in the art would still know how to use the nucleic acid

molecule, for instance, as a hybridization probe or a polymerase chain reaction (PCR) primer.

Mutations can also be introduced into the recombination site nucleotide sequences for enhancing site specific recombination or altering the specificities of the reactants, etc. Such mutations include, but are not limited to: recombination sites without translation stop codons that allow fusion proteins to be encoded; recombination sites recognized by the same proteins but differing in base sequence such that they react largely or exclusively with their homologous partners allowing multiple reactions to be contemplated; and mutations that prevent hairpin formation of recombination sites. Which particular reactions take place can be specified by which particular partners are present in the reaction mixture.

There are well known procedures for introducing specific mutations into nucleic acid sequences. A number of these are described in Ausubel, F.M. *et al.*, *Current Protocols in Molecular Biology*, Wiley Interscience, New York (1989-1996). Mutations can be designed into oligonucleotides, which can be used to modify existing cloned sequences, or in amplification reactions. Random mutagenesis can also be employed if appropriate selection methods are available to isolate the desired mutant DNA or RNA. The presence of the desired mutations can be confirmed by sequencing the nucleic acid by well known methods.

The following non-limiting methods can be used to modify or mutate a given nucleic acid molecule encoding a particular recombination site to provide mutated sites that can be used in the present invention:

1. By recombination of two parental DNA sequences by site-specific (e.g. attL and attR to give attP) or other (e.g. homologous) recombination mechanisms where the parental DNA segments contain one or more base alterations resulting in the final mutated nucleic acid molecule;
2. By mutation or mutagenesis (site-specific, PCR, random, spontaneous, etc) directly of the desired nucleic acid molecule;
3. By mutagenesis (site-specific, PCR, random, spontaneous, etc) of parental DNA sequences, which are recombined to generate a desired nucleic acid molecule;



4. By reverse transcription of an RNA encoding the desired core sequence;  
and

5. By *de novo* synthesis (chemical synthesis) of a sequence having the desired  
base changes, or random base changes followed by sequencing or  
functional analysis according to methods that are routine in the art.

The functionality of the mutant recombination sites can be demonstrated in  
ways that depend on the particular characteristic that is desired. For example, the  
lack of translation stop codons in a recombination site can be demonstrated by  
expressing the appropriate fusion proteins. Specificity of recombination between  
homologous partners can be demonstrated by introducing the appropriate  
molecules into *in vitro* reactions, and assaying for recombination products as  
described herein or known in the art. Other desired mutations in recombination  
sites might include the presence or absence of restriction sites, translation or  
transcription start signals, protein binding sites, particular coding sequences, and  
other known functionalities of nucleic acid base sequences. Genetic selection  
schemes for particular functional attributes in the recombination sites can be used  
according to known method steps. For example, the modification of sites to  
provide (from a pair of sites that do not interact) partners that do interact could  
be achieved by requiring deletion, via recombination between the sites, of a DNA  
sequence encoding a toxic substance. Similarly, selection for sites that remove  
translation stop sequences, the presence or absence of protein binding sites, etc.,  
can be easily devised by those skilled in the art.

Accordingly, the present invention also provides a nucleic acid molecule,  
comprising at least one DNA segment having at least one, and preferably at least  
two, engineered recombination site nucleotide sequences of the invention flanking  
a selectable marker and/or a desired DNA segment, wherein at least one of said  
recombination site nucleotide sequences has at least one engineered mutation that  
enhances recombination *in vitro* in the formation of a Cointegrate DNA or a  
Product DNA. Such engineered mutations may be in the core sequence of the  
recombination site nucleotide sequence of the invention; *see* U.S. Application Nos.  
08/486,139, filed June 7, 1995, 08/663,002, filed June 7, 1996 (now U.S. Patent  
No. 5,888,732), 09/005,476, filed January 12, 1998, and 09/177,387, filed

October 23, 1998, the disclosures of which are all incorporated herein by reference in their entireties.

While in the preferred embodiment the recombination sites differ in sequence and do not interact with each other, it is recognized that sites comprising the same sequence, which may interact with each other, can be manipulated or engineered to inhibit recombination with each other. Such conceptions are considered and incorporated herein. For example, a protein binding site (*e.g.*, an antibody-binding site, a histone-binding site, an enzyme-binding site, or a binding site for any nucleic acid molecule-binding protein) can be engineered adjacent to one of the sites. In the presence of the protein that recognizes the engineered site, the recombinase fails to access the site and another recombination site in the nucleic acid molecule is therefore used preferentially. In the cointegrate this site can no longer react since it has been changed, *e.g.*, from attB to attL. During or upon resolution of the cointegrate, the protein can be inactivated (*e.g.*, by antibody, heat or a change of buffer) and the second site can undergo recombination.

The nucleic acid molecules of the invention can have at least one mutation that confers at least one enhancement of said recombination, said enhancement selected from the group consisting of substantially (i) favoring integration; (ii) favoring recombination; (ii) relieving the requirement for host factors; (iii) increasing the efficiency of said Cointegrate DNA or Product DNA formation; (iv) increasing the specificity of said Cointegrate DNA or Product DNA formation; and (v) adding or deleting protein binding sites.

In other embodiments, the nucleic acid molecules of the invention may be PCR primer molecules, which comprise one or more of the recombination site sequences described herein or portions thereof, particularly those shown in Figure 9 (or sequences complementary to those shown in Figure 9), or mutants, fragments, variants or derivatives thereof, attached at the 3' end to a target-specific template sequence which specifically interacts with a target nucleic acid molecule which is to be amplified. Primer molecules according to this aspect of the invention may further comprise one or more, (*e.g.*, 1, 2, 3, 4, 5, 10, 20, 25, 50, 100, 500, 1000, or more) additional bases at their 5' ends, and preferably comprise one or more (particularly four or five) additional bases, which are preferably

guanines, at their 5' ends, to increase the efficiency of the amplification products incorporating the primer molecules in the recombinational cloning system of the invention. Such nucleic acid molecules and primers are described in detail in the examples herein, particularly in Examples 22-25.

5 Certain primers of the invention may comprise one or more nucleotide deletions in the *attB1*, *attB2*, *attP1*, *attP2*, *attL1*, *attL2*, *attR1* or *attR2* sequences as set forth in Figure 9. In one such aspect, for example, *attB2* primers may be constructed in which one or more of the first four nucleotides at the 5' end of the *attB2* sequence shown in Figure 9 have been deleted. Primers according to this aspect of the invention may therefore have the sequence:

(*attB2*(-1)): CCCAGCTTTCTTGTACAAAGTGGTnnnnnnnnnnnn . . . n

(*attB2*(-2)): CCAGCTTTCTTGTACAAAGTGGTnnnnnnnnnnnn . . . n

(*attB2*(-3)): CAGCTTTCTTGTACAAAGTGGTnnnnnnnnnnnn . . . n

(*attB2*(-4)): AGCTTTCTTGTACAAAGTGGTnnnnnnnnnnnn . . . n,

15 wherein "nnnnnnnnnnnn . . . n" at the 3' end of the primer represents a target-specific sequence of any length, for example from one base up to all of the bases of a target nucleic acid molecule (*e.g.*, a gene) or a portion thereof, the sequence and length which will depend upon the identity of the target nucleic acid molecule which is to be amplified.

20 The primer nucleic acid molecules according to this aspect of the invention may be produced synthetically by attaching the recombination site sequences depicted in Figure 9, or portions thereof, to the 5' end of a standard PCR target-specific primer according to methods that are well-known in the art. Alternatively, additional primer nucleic acid molecules of the invention may be produced synthetically by adding one or more nucleotide bases, which preferably correspond to one or more, preferably five or more, and more preferably six or more, contiguous nucleotides of the *att* nucleotide sequences described herein (*see, e.g.*, Example 20 herein; *see also* U.S. Application Nos. 08/663,002, filed June 7, 1996 (now U.S. Patent No. 5,888,732), 09/005,476, filed January 12, 1998, and 09/177,387, filed October 23, 1998, the disclosures of which are all incorporated herein by reference in their entireties), to the 5' end of a standard PCR target-specific primer according to methods that are well-known in the art, to provide

primers having the specific nucleotide sequences described herein. As noted above, primer nucleic acid molecules according to this aspect of the invention may also optionally comprise one, two, three, four, five, or more additional nucleotide bases at their 5' ends, and preferably will comprise four or five guanines at their 5' ends. In one particularly preferred such aspect, the primer nucleic acid molecules of the invention may comprise one or more, preferably five or more, more preferably six or more, still more preferably 6-18 or 6-25, and most preferably 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25, contiguous nucleotides or bp of the *attB1* or *attB2* nucleotide sequences depicted in Figure 9 (or nucleotides complementary thereto), linked to the 5' end of a target-specific (*e.g.*, a gene-specific) primer molecule. Primer nucleic acid molecules according to this aspect of the invention include, but are not limited to, *attB1*- and *attB2*-derived primer nucleic acid molecules having the following nucleotide sequences:

ACAAGTTTGTACAAAAAGCAGGCT-nnnnnnnnnnnnn . . . n  
ACCACTTTGTACAAGAAAGCTGGGT-nnnnnnnnnnnnn . . . n  
TGTACAAAAAGCAGGCT-nnnnnnnnnnnnn . . . n  
TGTACAAGAAAGCTGGGT-nnnnnnnnnnnnn . . . n  
ACAAAAAGCAGGCT-nnnnnnnnnnnnn . . . n  
ACAAGAAAGCTGGGT-nnnnnnnnnnnnn . . . n  
AAAAAGCAGGCT-nnnnnnnnnnnnn . . . n  
AGAAAGCTGGGT-nnnnnnnnnnnnn . . . n  
AAAAGCAGGCT-nnnnnnnnnnnnn . . . n  
GAAAGCTGGGT-nnnnnnnnnnnnn . . . n  
AAAGCAGGCT-nnnnnnnnnnnnn . . . n  
AAAGCTGGGT-nnnnnnnnnnnnn . . . n  
AAGCAGGCT-nnnnnnnnnnnnn . . . n  
AAGCTGGGT-nnnnnnnnnnnnn . . . n  
AGCAGGCT-nnnnnnnnnnnnn . . . n  
AGCTGGGT-nnnnnnnnnnnnn . . . n  
GCAGGCT-nnnnnnnnnnnnn . . . n  
GCTGGGT-nnnnnnnnnnnnn . . . n

CAGGCT-nnnnnnnnnnnnn . . . n

CTGGGT-nnnnnnnnnnnnn . . . n,

wherein "nnnnnnnnnnnn . . . n" at the 3' end of the primer represents a target-specific sequence of any length, for example from one base up to all of the bases of a target nucleic acid molecule (*e.g.*, a gene) or a portion thereof, the sequence and length which will depend upon the identity of the target nucleic acid molecule which is to be amplified.

Of course, it will be apparent to one of ordinary skill from the teachings contained herein that additional primer nucleic acid molecules analogous to those specifically described herein may be produced using one or more, preferably five or more, more preferably six or more, still more preferably ten or more, 15 or more, 20 or more, 25 or more, 30 or more, etc. (through to and including all) of the contiguous nucleotides or bp of the *attP1*, *attP2*, *attL1*, *attL2*, *attR1* or *attR2* nucleotide sequences depicted in Figure 9 (or nucleotides complementary thereto), linked to the 5' end of a target-specific (*e.g.*, a gene-specific) primer molecule. As noted above, such primer nucleic acid molecules may optionally further comprise one, two, three, four, five, or more additional nucleotide bases at their 5' ends, and preferably will comprise four guanines at their 5' ends. Other primer molecules comprising the *attB1*, *attB2*, *attP1*, *attP2*, *attL1*, *attL2*, *attR1* and *attR2* sequences depicted in Figure 9, or portions thereof, may be made by one of ordinary skill without resorting to undue experimentation in accordance with the guidance provided herein.

The primers of the invention described herein are useful in producing PCR fragments having a nucleic acid molecule of interest flanked at each end by a recombination site sequence (as described in detail below in Example 9), for use in cloning of PCR-amplified DNA fragments using the recombination system of the invention (as described in detail below in Examples 8, 19 and 21-25).

### **Vectors**

The invention also relates to vectors comprising one or more of the nucleic acid molecules of the invention, as described herein. In accordance with the invention, any vector may be used to construct the vectors of the invention. In

particular, vectors known in the art and those commercially available (and variants or derivatives thereof) may in accordance with the invention be engineered to include one or more nucleic acid molecules encoding one or more recombination sites (or portions thereof), or mutants, fragments, or derivatives thereof, for use in the methods of the invention. Such vectors may be obtained from, for example, Vector Laboratories Inc., Invitrogen, Promega, Novagen, New England Biolabs, Clontech, Roche, Pharmacia, EpiCenter, OriGenes Technologies Inc., Stratagene, Perkin Elmer, Pharmingen, Life Technologies, Inc., and Research Genetics. Such vectors may then for example be used for cloning or subcloning nucleic acid molecules of interest. General classes of vectors of particular interest include prokaryotic and/or eukaryotic cloning vectors, Expression Vectors, fusion vectors, two-hybrid or reverse two-hybrid vectors, shuttle vectors for use in different hosts, mutagenesis vectors, transcription vectors, vectors for receiving large inserts and the like.

Other vectors of interest include viral origin vectors (M13 vectors, bacterial phage  $\lambda$  vectors, bacteriophage P1 vectors, adenovirus vectors, herpesvirus vectors, retrovirus vectors, phage display vectors, combinatorial library vectors), high, low, and adjustable copy number vectors, vectors which have compatible replicons for use in combination in a single host (pACYC184 and pBR322) and eukaryotic episomal replication vectors (pCDM8).

Particular vectors of interest include prokaryotic Expression Vectors such as pcDNA II, pSL301, pSE280, pSE380, pSE420, pTrcHisA, B, and C, pRSET A, B, and C (Invitrogen, Inc.), pGEMEX-1, and pGEMEX-2 (Promega, Inc.), the pET vectors (Novagen, Inc.), pTrc99A, pKK223-3, the pGEX vectors, pEZZ18, pRIT2T, and pMC1871 (Pharmacia, Inc.), pKK233-2 and pKK388-1 (Clontech, Inc.), and pProEx-HT (Life Technologies, Inc.) and variants and derivatives thereof. Destination Vectors can also be made from eukaryotic Expression Vectors such as pFastBac, pFastBac HT, pFastBac DUAL, pSFV, and pTet-Splice (Life Technologies, Inc.), pEUK-C1, pPUR, pMAM, pMAMneo, pBI101, pBI121, pDR2, pCMVEBNA, and pYACneo (Clontech), pSVK3, pSVL, pMSG, pCH110, and pKK232-8 (Pharmacia, Inc.), p3'SS, pXT1, pSG5, pBac, pMbac, pMC1neo, and pOG44 (Stratagene, Inc.), and pYES2, pAC360, pBlueBacHis A,

B, and C, pVL1392, pBsueBacIII, pCDM8, pcDNA1, pZeoSV, pcDNA3 pREP4, pCEP4, and pEBVHis (Invitrogen, Inc.) and variants or derivatives thereof.

Other vectors of particular interest include pUC18, pUC19, pBlueScript, pSPORT, cosmids, phagemids, YACs (yeast artificial chromosomes), BACs  
 5 (bacterial artificial chromosomes), MACs (mammalian artificial chromosomes), pQE70, pQE60, pQE9 (Quiagen), pBS vectors, PhageScript vectors, BlueScript vectors, pNH8A, pNH16A, pNH18A, pNH46A (Stratagene), pcDNA3 (Invitrogen), pGEX, pTrsfus, pTrc99A, pET-5, pET-9, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia), pSPORT1, pSPORT2, pCMVSPORT2.0 and pSV-SPORT1 (Life Technologies, Inc.) and variants or derivatives thereof.

Additional vectors of interest include pTrxFus, pThioHis, pLEX, pTrcHis, pTrcHis2, pRSET, pBlueBacHis2, pcDNA3.1/His, pcDNA3.1(-)/Myc-His, pSecTag, pEBVHis, pPIC9K, pPIC3.5K, pAO815, pPICZ, pPICZ $\alpha$ , pGAPZ, pGAPZ $\alpha$ , pBlueBac4.5, pBlueBacHis2, pMelBac, pSinRep5, pSinHis, pIND,  
 15 pIND(SP1), pVgRXR, pcDNA2.1, pYES2, pZErO1.1, pZErO-2.1, pCR-Blunt, pSE280, pSE380, pSE420, pVL1392, pVL1393, pCDM8, pcDNA1.1, pcDNA1.1/Amp, pcDNA3.1, pcDNA3.1/Zeo, pSe,SV2, pRc/CMV2, pRc/RSV, pREP4, pREP7, pREP8, pREP9, pREP10, pCEP4, pEBVHis, pCR3.1, pCR2.1, pCR3.1-Uni, and pCRBac from Invitrogen;  $\lambda$ ExCell,  $\lambda$ gt11, pTrc99A, pKK223-3, pGEX-1 $\lambda$ T, pGEX-2T, pGEX-2TK, pGEX-4T-1, pGEX-4T-2, pGEX-4T-3,  
 20 pGEX-3X, pGEX-5X-1, pGEX-5X-2, pGEX-5X-3, pEZZ18, pRIT2T, pMC1871, pSVK3, pSVL, pMSG, pCH110, pKK232-8, pSL1180, pNEO, and pUC4K from Pharmacia; pSCREEN-1b(+), pT7Blue(R), pT7Blue-2, pCITE-4abc(+), pOCUS-2, pTag, pET-32 LIC, pET-30 LIC, pBAC-2cp LIC, pBACgus-2cp LIC, pT7Blue-2 LIC, pT7Blue-2,  $\lambda$ SCREEN-1,  $\lambda$ BlueSTAR, pET-3abcd,  
 25 pET-7abc, pET9abcd, pET11abcd, pET12abc, pET-14b, pET-15b, pET-16b, pET-17b-pET-17xb, pET-19b, pET-20b(+), pET-21abcd(+), pET-22b(+), pET-23abcd(+), pET-24abcd(+), pET-25b(+), pET-26b(+), pET-27b(+), pET-28abc(+), pET-29abc(+), pET-30abc(+), pET-31b(+), pET-32abc(+), pET-33b(+), pBAC-1, pBACgus-1, pBAC4x-1, pBACgus4x-1, pBAC-3cp, pBACgus-2cp, pBACsurf-1, plg, Signal plg, pYX, Selecta Vecta-Neo, Selecta Vecta - Hyg, and Selecta Vecta - Gpt from Novagen; pLexA, pB42AD, pGBT9, pAS2-1,

pGAD424, pACT2, pGAD GL, pGAD GH, pGAD10, pGilda, pEZM3, pEGFP, pEGFP-1, pEGFP-N, pEGFP-C, pEBFP, pGFPuv, pGFP, p6xHis-GFP, pSEAP2-Basic, pSEAP2-Contral, pSEAP2-Promoter, pSEAP2-Enhancer, p $\beta$ gal-Basic, p $\beta$ gal-Control, p $\beta$ gal-Promoter, p $\beta$ gal-Enhancer, pCMV $\beta$ , pTet-Off, pTet-On, pTK-Hyg, pRetro-Off, pRetro-On, pIRES1neo, pIRES1hyg, pLXSN, pLNCX, pLAPSN, pMAMneo, pMAMneo-CAT, pMAMneo-LUC, pPUR, pSV2neo, pYEX4T-1/2/3, pYEX-S1, pBacPAK-His, pBacPAK8/9, pAcUW31, BacPAK6, pTriplEx,  $\lambda$ gt10,  $\lambda$ gt11, pWE15, and  $\lambda$ TriplEx from Clontech; Lambda ZAP II, pBK-CMV, pBK-RSV, pBluescript II KS +/-, pBluescript II SK +/-, pAD-GAL4, pBD-GAL4 Cam, pSurfscrip, Lambda FIX II, Lambda DASH, Lambda EMBL3, Lambda EMBL4, SuperCos, pCR-Script Amp, pCR-Script Cam, pCR-Script Direct, pBS +/-, pBC KS +/-, pBC SK +/-, Phagescript, pCAL-n-EK, pCAL-n, pCAL-c, pCAL-kc, pET-3abcd, pET-11abcd, pSPUTK, pESP-1, pCMVLacI, pOPRSVI/MCS, pOPI3 CAT, pXT1, pSG5, pPbac, pMbac, pMC1neo, pMC1neo Poly A, pOG44, pOG45, pFRT $\beta$ GAL, pNEO $\beta$ GAL, pRS403, pRS404, pRS405, pRS406, pRS413, pRS414, pRS415, and pRS416 from Stratagene.

Two-hybrid and reverse two-hybrid vectors of particular interest include pPC86, pDBLeu, pDBTrp, pPC97, p2.5, pGAD1-3, pGAD10, pAct, pACT2, pGADGL, pGADGH, pAS2-1, pGAD424, pGBT8, pGBT9, pGAD-GAL4, pLexA, pBD-GAL4, pHISi, pHISi-1, placZi, pB42AD, pDG202, pJK202, pJG4-5, pNLexA, pYESTrp and variants or derivatives thereof.

Yeast Expression Vectors of particular interest include pESP-1, pESP-2, pESC-His, pESC-Trp, pESC-URA, pESC-Leu (Stratagene), pRS401, pRS402, pRS411, pRS412, pRS421, pRS422, and variants or derivatives thereof.

According to the invention, the vectors comprising one or more nucleic acid molecules encoding one or more recombination sites, or mutants, variants, fragments, or derivatives thereof, may be produced by one of ordinary skill in the art without resorting to undue experimentation using standard molecular biology methods. For example, the vectors of the invention may be produced by introducing one or more of the nucleic acid molecules encoding one or more recombination sites (or mutants, fragments, variants or derivatives thereof) into one or more of the vectors described herein, according to the methods described,



for example, in Maniatis *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (1982). In a related aspect of the invention, the vectors may be engineered to contain, in addition to one or more nucleic acid molecules encoding one or more recombination sites (or portions thereof), one or more additional physical or functional nucleotide sequences, such as those encoding one or more multiple cloning sites, one or more transcription termination sites, one or more transcriptional regulatory sequences (*e.g.*, one or more promoters, enhancers, or repressors), one or more selection markers or modules, one or more genes or portions of genes encoding a protein or polypeptide of interest, one or more translational signal sequences, one or more nucleotide sequences encoding a fusion partner protein or peptide (*e.g.*, GST, His<sub>6</sub> or thioredoxin), one or more origins of replication, and one or more 5' or 3' polynucleotide tails (particularly a poly-G tail). According to this aspect of the invention, the one or more recombination site nucleotide sequences (or portions thereof) may optionally be operably linked to the one or more additional physical or functional nucleotide sequences described herein.

Preferred vectors according to this aspect of the invention include, but are not limited to: pENTR1A (Figures 10A and 10B), pENTR2B (Figures 11A and 11B), pENTR3C (Figures 12A and 12B), pENTR4 (Figures 13A and 13B), pENTR5 (Figures 14A and 14B), pENTR6 (Figures 15A and 15B), pENTR7 (Figures 16A and 16B), pENTR8 (Figures 17A and 17B), pENTR9 (Figures 18A and 18B), pENTR10 (Figures 19A and 19B), pENTR11 (Figures 20A and 20B), pDEST1 (Figures 21A-D), pDEST2 (Figure 22A-D), pDEST3 (Figure 23A-D), pDEST4 (Figure 24A-D), pDEST5 (Figure 25A-D), pDEST6 (Figure 26A-D), pDEST7 (Figure 27A-C), pDEST8 (Figure 28A-D), pDEST9 (Figure 29A-E), pDEST10 (Figure 30A-D), pDEST11 (Figure 31A-D), pDEST12.2 (also known as pDEST12) (Figure 32A-D), pDEST13 (Figure 33A-C), pDEST14 (Figure 34A-D), pDEST15 (Figure 35A-D), pDEST16 (Figure 36A-D), pDEST17 (Figure 37A-D), pDEST18 (Figure 38A-D), pDEST19 (Figure 39A-D), pDEST20 (Figure 40A-D), pDEST21 (Figure 41A-E), pDEST22 (Figure 42A-D), pDEST23 (Figure 43A-D), pDEST24 (Figure 44A-D), pDEST25 (Figure 45A-D), pDEST26 (Figure 46A-D), pDEST27 (Figure 47A-D), pEXP501 (also known

as pCMVSPORT6) (Figure 48A-B), pDONR201 (also known as pENTR21 attP vector or pAttPkan Donor Vector) (Figure 49), pDONR202 (Figure 50), pDONR203 (also known as pEZ15812) (Figure 51), pDONR204 (Figure 52), pDONR205 (Figure 53), pDONR206 (also known as pENTR22 attP vector or pAttPgen Donor Vector) (Figure 54), pMAB58 (Figure 87), pMAB62 (Figure 88), pDEST28 (Figure 90), pDEST29 (Figure 91), pDEST30 (Figure 92), pDEST31 (Figure 93), pDEST32 (Figure 94), pDEST33 (Figure 95), pDEST34 (Figure 96), pDONR207 (Figure 97), pMAB85 (Figure 98), pMAB86 (Figure 99), and fragments, mutants, variants, and derivatives thereof. However, it will be understood by one of ordinary skill that the present invention also encompasses other vectors not specifically designated herein, which comprise one or more of the isolated nucleic acid molecules of the invention encoding one or more recombination sites or portions thereof (or mutants, fragments, variants or derivatives thereof), and which may further comprise one or more additional physical or functional nucleotide sequences described herein which may optionally be operably linked to the one or more nucleic acid molecules encoding one or more recombination sites or portions thereof. Such additional vectors may be produced by one of ordinary skill according to the guidance provided in the present specification.

### ***Polymerases***

Preferred polypeptides having reverse transcriptase activity (*i.e.*, those polypeptides able to catalyze the synthesis of a DNA molecule from an RNA template) for use in accordance with the present invention include, but are not limited to Moloney Murine Leukemia Virus (M-MLV) reverse transcriptase, Rous Sarcoma Virus (RSV) reverse transcriptase, Avian Myeloblastosis Virus (AMV) reverse transcriptase, Rous Associated Virus (RAV) reverse transcriptase, Myeloblastosis Associated Virus (MAV) reverse transcriptase, Human Immunodeficiency Virus (HIV) reverse transcriptase, retroviral reverse transcriptase, retrotransposon reverse transcriptase, hepatitis B reverse transcriptase, cauliflower mosaic virus reverse transcriptase and bacterial reverse transcriptase. Particularly preferred are those polypeptides having reverse

transcriptase activity that are also substantially reduced in RNase H activity (*i.e.*, “RNase H” polypeptides). By a polypeptide that is “substantially reduced in RNase H activity” is meant that the polypeptide has less than about 20%, more preferably less than about 15%, 10% or 5%, and most preferably less than about 2%, of the RNase H activity of a wildtype or RNase H<sup>-</sup> enzyme such as wildtype M-MLV reverse transcriptase. The RNase H activity may be determined by a variety of assays, such as those described, for example, in U.S. Patent No. 5,244,797, in Kotewicz, M.L. *et al.*, *Nucl. Acids Res.* 16:265 (1988) and in Gerard, G.F., *et al.*, *FOCUS* 14(5):91 (1992), the disclosures of all of which are fully incorporated herein by reference. Suitable RNase H<sup>-</sup> polypeptides for use in the present invention include, but are not limited to, M-MLV H<sup>-</sup> reverse transcriptase, RSV H<sup>-</sup> reverse transcriptase, AMV H<sup>-</sup> reverse transcriptase, RAV H<sup>-</sup> reverse transcriptase, MAV H<sup>-</sup> reverse transcriptase, HIV H<sup>-</sup> reverse transcriptase, THERMOSCRIPT™ reverse transcriptase and THERMOSCRIPT™ II reverse transcriptase, and SUPERScript™ I reverse transcriptase and SUPERScript™ II reverse transcriptase, which are obtainable, for example, from Life Technologies, Inc. (Rockville, Maryland). See generally published PCT application WO 98/47912.

Other polypeptides having nucleic acid polymerase activity suitable for use in the present methods include thermophilic DNA polymerases such as DNA polymerase I, DNA polymerase III, Klenow fragment, T7 polymerase, and T5 polymerase, and thermostable DNA polymerases including, but not limited to, *Thermus thermophilus* (*Tth*) DNA polymerase, *Thermus aquaticus* (*Taq*) DNA polymerase, *Thermotoga neopolitana* (*Tne*) DNA polymerase, *Thermotoga maritima* (*Tma*) DNA polymerase, *Thermococcus litoralis* (*Tli* or VENT®) DNA polymerase, *Pyrococcus furiosus* (*Pfu*) DNA polymerase, *Pyrococcus* species GB-D (or DEEPVENT®) DNA polymerase, *Pyrococcus woosii* (*Pwo*) DNA polymerase, *Bacillus sterothermophilus* (*Bst*) DNA polymerase, *Sulfolobus acidocaldarius* (*Sac*) DNA polymerase, *Thermoplasma acidophilum* (*Tac*) DNA polymerase, *Thermus flavus* (*Tfl/Tub*) DNA polymerase, *Thermus ruber* (*Tru*) DNA polymerase, *Thermus brockianus* (DYNAZYME®) DNA polymerase, *Methanobacterium thermoautotrophicum* (*Mth*) DNA polymerase, and mutants,

variants and derivatives thereof. Such polypeptides are available commercially, for example from Life Technologies, Inc. (Rockville, MD), New Englan BioLabs (Beverly, MA), and Sigma/Aldrich (St. Louis, MO).

### 5      *Host Cells*

The invention also relates to host cells comprising one or more of the nucleic acid molecules or vectors of the invention, particularly those nucleic acid molecules and vectors described in detail herein. Representative host cells that may be used according to this aspect of the invention include, but are not limited to, bacterial cells, yeast cells, plant cells and animal cells. Preferred bacterial host cells include *Escherichia* spp. cells (particularly *E. coli* cells and most particularly *E. coli* strains DH10B, Stbl2, DH5 $\alpha$ , DB3, DB3.1 (preferably *E. coli* LIBRARY EFFICIENCY $\circledR$  DB3.1<sup>TM</sup> Competent Cells; Life Technologies, Inc., Rockville, MD), DB4 and DB5; see U.S. Provisional Application No. 60/122,392, filed on March 2, 1999, the disclosure of which is incorporated by reference herein in its entirety), *Bacillus* spp. cells (particularly *B. subtilis* and *B. megaterium* cells), *Streptomyces* spp. cells, *Erwinia* spp. cells, *Klebsiella* spp. cells, *Serratia* spp. cells (particularly *S. marcessans* cells), *Pseudomonas* spp. cells (particularly *P. aeruginosa* cells), and *Salmonella* spp. cells (particularly *S. typhimurium* and *S. typhi* cells). Preferred animal host cells include insect cells (most particularly *Drosophila melanogaster* cells, *Spodoptera frugiperda* Sf9 and Sf21 cells and *Trichoplusa* High-Five cells), nematode cells (particularly *C. elegans* cells), avian cells, amphibian cells (particularly *Xenopus laevis* cells), reptilian cells, and mammalian cells (most particularly CHO, COS, VERO, BHK and human cells). Preferred yeast host cells include *Saccharomyces cerevisiae* cells and *Pichia pastoris* cells. These and other suitable host cells are available commercially, for example from Life Technologies, Inc. (Rockville, Maryland), American Type Culture Collection (Manassas, Virginia), and Agricultural Research Culture Collection (NRRL; Peoria, Illinois).

30      Methods for introducing the nucleic acid molecules and/or vectors of the invention into the host cells described herein, to produce host cells comprising one or more of the nucleic acid molecules and/or vectors of the invention, will be

familiar to those of ordinary skill in the art. For instance, the nucleic acid molecules and/or vectors of the invention may be introduced into host cells using well known techniques of infection, transduction, transfection, and transformation. The nucleic acid molecules and/or vectors of the invention may be introduced  
5 alone or in conjunction with other the nucleic acid molecules and/or vectors. Alternatively, the nucleic acid molecules and/or vectors of the invention may be introduced into host cells as a precipitate, such as a calcium phosphate precipitate, or in a complex with a lipid. Electroporation also may be used to introduce the nucleic acid molecules and/or vectors of the invention into a host. Likewise, such  
10 molecules may be introduced into chemically competent cells such as *E. coli*. If the vector is a virus, it may be packaged *in vitro* or introduced into a packaging cell and the packaged virus may be transduced into cells. Hence, a wide variety of techniques suitable for introducing the nucleic acid molecules and/or vectors of the invention into cells in accordance with this aspect of the invention are well  
15 known and routine to those of skill in the art. Such techniques are reviewed at length, for example, in Sambrook, J., *et al.*, *Molecular Cloning, a Laboratory Manual, 2nd Ed.*, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, pp. 16.30-16.55 (1989), Watson, J.D., *et al.*, *Recombinant DNA, 2nd Ed.*, New York: W.H. Freeman and Co., pp. 213-234 (1992), and Winnacker, E.-L.,  
20 *From Genes to Clones*, New York: VCH Publishers (1987), which are illustrative of the many laboratory manuals that detail these techniques and which are incorporated by reference herein in their entireties for their relevant disclosures.

### ***Polypeptides***

25 In another aspect, the invention relates to polypeptides encoded by the nucleic acid molecules of the invention (including polypeptides and amino acid sequences encoded by all possible reading frames of the nucleic acid molecules of the invention), and to methods of producing such polypeptides. Polypeptides of the present invention include purified or isolated natural products, products of  
30 chemical synthetic procedures, and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, insect, mammalian, avian and higher plant cells.

The polypeptides of the invention may be produced by synthetic organic chemistry, and are preferably produced by standard recombinant methods, employing one or more of the host cells of the invention comprising the vectors or isolated nucleic acid molecules of the invention. According to the invention, polypeptides are produced by cultivating the host cells of the invention (which comprise one or more of the nucleic acid molecules of the invention, preferably contained within an Expression Vector) under conditions favoring the expression of the nucleotide sequence contained on the nucleic acid molecule of the invention, such that the polypeptide encoded by the nucleic acid molecule of the invention is produced by the host cell. As used herein, "conditions favoring the expression of the nucleotide sequence" or "conditions favoring the production of a polypeptide" include optimal physical (*e.g.*, temperature, humidity, etc.) and nutritional (*e.g.*, culture medium, ionic) conditions required for production of a recombinant polypeptide by a given host cell. Such optimal conditions for a variety of host cells, including prokaryotic (bacterial), mammalian, insect, yeast, and plant cells will be familiar to one of ordinary skill in the art, and may be found, for example, in Sambrook, J., *et al.*, *Molecular Cloning, A Laboratory Manual, 2nd Ed.*, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, (1989), Watson, J.D., *et al.*, *Recombinant DNA, 2nd Ed.*, New York: W.H. Freeman and Co., and Winnacker, E.-L., *From Genes to Clones*, New York: VCH Publishers (1987).

In some aspects, it may be desirable to isolate or purify the polypeptides of the invention (*e.g.*, for production of antibodies as described below), resulting in the production of the polypeptides of the invention in isolated form. The polypeptides of the invention can be recovered and purified from recombinant cell cultures by well-known methods of protein purification that are routine in the art, including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. For example, His6 or GST fusion tags on polypeptides made by the methods of the invention may be isolated using appropriate affinity chromatography matrices which bind polypeptides bearing

His6 or GST tags, as will be familiar to one of ordinary skill in the art. Polypeptides of the present invention include naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes.

Isolated polypeptides of the invention include those comprising the amino acid sequences encoded by one or more of the reading frames of the polynucleotides comprising one or more of the recombination site-encoding nucleic acid molecules of the invention, including those encoding *attB1*, *attB2*, *attP1*, *attP2*, *attL1*, *attL2*, *attR1* and *attR2* having the nucleotide sequences set forth in Figure 9 (or nucleotide sequences complementary thereto), or fragments, variants, mutants and derivatives thereof; the complete amino acid sequences encoded by the polynucleotides contained in the deposited clones described herein; the amino acid sequences encoded by polynucleotides which hybridize under stringent hybridization conditions to polynucleotides having the nucleotide sequences encoding the recombination site sequences of the invention as set forth in Figure 9 (or a nucleotide sequence complementary thereto); or a peptide or polypeptide comprising a portion or a fragment of the above polypeptides. The invention also relates to additional polypeptides having one or more additional amino acids linked (typically by peptidyl bonds to form a nascent polypeptide) to the polypeptides encoded by the recombination site nucleotide sequences or the deposited clones. Such additional amino acid residues may comprise one or more functional peptide sequences, for example one or more fusion partner peptides (*e.g.*, GST, His<sub>6</sub>, Trx, etc.) and the like.

As used herein, the terms "protein," "peptide," "oligopeptide" and "polypeptide" are considered synonymous (as is commonly recognized) and each term can be used interchangeably as the context requires to indicate a chain of two or more amino acids, preferably five or more amino acids, or more preferably ten

or more amino acids, coupled by (a) peptidyl linkage(s), unless otherwise defined in the specific contexts below. As is commonly recognized in the art, all polypeptide formulas or sequences herein are written from left to right and in the direction from amino terminus to carboxy terminus.

5 It will be recognized by those of ordinary skill in the art that some amino acid sequences of the polypeptides of the invention can be varied without significant effect on the structure or function of the polypeptides. If such differences in sequence are contemplated, it should be remembered that there will be critical areas on the protein which determine structure and activity. In general, it is possible to replace residues which form the tertiary structure, provided that  
10 residues performing a similar function are used. In other instances, the type of residue may be completely unimportant if the alteration occurs at a non-critical region of the polypeptide.

Thus, the invention further includes variants of the polypeptides of the invention, including allelic variants, which show substantial structural homology  
15 to the polypeptides described herein, or which include specific regions of these polypeptides such as the portions discussed below. Such mutants may include deletions, insertions, inversions, repeats, and type substitutions (for example, substituting one hydrophilic residue for another, but not strongly hydrophilic for  
20 strongly hydrophobic as a rule). Small changes or such "neutral" or "conservative" amino acid substitutions will generally have little effect on activity.

Typical conservative substitutions are the replacements, one for another, among the aliphatic amino acids Ala, Val, Leu and Ile; interchange of the hydroxylated residues Ser and Thr; exchange of the acidic residues Asp and Glu;  
25 substitution between the amidated residues Asn and Gln; exchange of the basic residues Lys and Arg; and replacements among the aromatic residues Phe and Tyr.

Thus, the fragment, derivative or analog of the polypeptides of the invention, such as those comprising peptides encoded by the recombination site nucleotide sequences described herein, may be (i) one in which one or more of the amino acid  
30 residues are substituted with a conservative or non-conservative amino acid residue (preferably a conservative amino acid residue), and such substituted amino acid residue may be encoded by the genetic code or may be an amino acid (*e.g.*,



desmosine, citrulline, ornithine, etc.) that is not encoded by the genetic code; (ii) one in which one or more of the amino acid residues includes a substituent group (e.g., a phosphate, hydroxyl, sulfate or other group) in addition to the normal "R" group of the amino acid; (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which additional amino acids are fused to the mature polypeptide, such as an immunoglobulin Fc region peptide, a leader or secretory sequence, a sequence which is employed for purification of the mature polypeptide (such as GST) or a proprotein sequence. Such fragments, derivatives and analogs are intended to be encompassed by the present invention, and are within the scope of those skilled in the art from the teachings herein and the state of the art at the time of invention.

The polypeptides of the present invention are preferably provided in an isolated form, and preferably are substantially purified. Recombinantly produced versions of the polypeptides of the invention can be substantially purified by the one-step method described in Smith and Johnson, *Gene* 67:31-40 (1988). As used herein, the term "substantially purified" means a preparation of an individual polypeptide of the invention wherein at least 50%, preferably at least 60%, 70%, or 75% and more preferably at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% (by mass) of contaminating proteins (*i.e.*, those that are not the individual polypeptides described herein or fragments, variants, mutants or derivatives thereof) have been removed from the preparation.

The polypeptides of the present invention include those which are at least about 50% identical, at least 60% identical, at least 65% identical, more preferably at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical, to the polypeptides described herein. For example, preferred *attB1*-containing polypeptides of the invention include those that are at least about 50% identical, at least 60% identical, at least 65% identical, more preferably at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical,

to the polypeptide(s) encoded by the three reading frames of a polynucleotide comprising a nucleotide sequence of *attB1* having a nucleic acid sequence as set forth in Figure 9 (or a nucleic acid sequence complementary thereto), to a polypeptide encoded by a polynucleotide contained in the deposited cDNA clones described herein, or to a polypeptide encoded by a polynucleotide hybridizing under stringent conditions to a polynucleotide comprising a nucleotide sequence of *attB1* having a nucleic acid sequence as set forth in Figure 9 (or a nucleic acid sequence complementary thereto). Analogous polypeptides may be prepared that are at least about 65% identical, more preferably at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical, to the *attB2*, *attP1*, *attP2*, *attL1*, *attL2*, *attR1* and *attR2* polypeptides of the invention as depicted in Figure 9. The present polypeptides also include portions or fragments of the above-described polypeptides with at least 5, 10, 15, 20, or 25 amino acids.

By a polypeptide having an amino acid sequence at least, for example, 65% "identical" to a reference amino acid sequence of a given polypeptide of the invention is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to 35 amino acid alterations per each 100 amino acids of the reference amino acid sequence of a given polypeptide of the invention. In other words, to obtain a polypeptide having an amino acid sequence at least 65% identical to a reference amino acid sequence, up to 35% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 35% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino (N-) or carboxy (C-) terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence. As a practical matter, whether a given amino acid sequence is, for example, at least 65% identical to the amino acid sequence of a given polypeptide of the invention can be determined

conventionally using known computer programs such as those described above for nucleic acid sequence identity determinations, or more preferably using the CLUSTAL W program (Thompson, J.D., *et al.*, *Nucleic Acids Res.* 22:4673-4680 (1994)).

5       The polypeptides of the present invention can be used as molecular weight markers on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art. In addition, as described in detail below, the polypeptides of the present invention can be used to raise polyclonal and monoclonal antibodies which are useful in a variety of assays for detecting  
10       protein expression, localization, detection of interactions with other molecules, or for the isolation of a polypeptide (including a fusion polypeptide) of the invention.

      In another aspect, the present invention provides a peptide or polypeptide comprising an epitope-bearing portion of a polypeptide of the invention, which may be used to raise antibodies, particularly monoclonal antibodies, that bind  
15       specifically to a one or more of the polypeptides of the invention. The epitope of this polypeptide portion is an immunogenic or antigenic epitope of a polypeptide of the invention. An "immunogenic epitope" is defined as a part of a protein that elicits an antibody response when the whole protein is the immunogen. These immunogenic epitopes are believed to be confined to a few loci on the molecule.  
20       On the other hand, a region of a protein molecule to which an antibody can bind is defined as an "antigenic epitope." The number of immunogenic epitopes of a protein generally is less than the number of antigenic epitopes (*see, e.g.*, Geysen *et al.*, *Proc. Natl. Acad. Sci. USA* 81:3998- 4002 (1983)).

      As to the selection of peptides or polypeptides bearing an antigenic epitope  
25       (*i.e.*, that contain a region of a protein molecule to which an antibody can bind), it is well-known in the art that relatively short synthetic peptides that mimic part of a protein sequence are routinely capable of eliciting an antiserum that reacts with the partially mimicked protein (*see, e.g.*, Sutcliffe, J.G., *et al.*, *Science* 219:660-666 (1983)). Peptides capable of eliciting protein-reactive sera are  
30       frequently represented in the primary sequence of a protein, can be characterized by a set of simple chemical rules, and are not confined to the immunodominant regions of intact proteins (*i.e.*, immunogenic epitopes) or to the amino or carboxy

termini. Peptides that are extremely hydrophobic and those of six or fewer residues generally are ineffective at inducing antibodies that bind to the mimicked protein; longer peptides, especially those containing proline residues, usually are effective (Sutcliffe, J.G., *et al.*, *Science* 219:660-666 (1983)).

5        Epitope-bearing peptides and polypeptides of the invention designed according to the above guidelines preferably contain a sequence of at least five, more preferably at least seven or more amino acids contained within the amino acid sequence of a polypeptide of the invention. However, peptides or polypeptides comprising a larger portion of an amino acid sequence of a polypeptide of the invention, containing about 30 to about 50 amino acids, or any length up to and including the entire amino acid sequence of a given polypeptide of the invention, also are considered epitope-bearing peptides or polypeptides of the invention and also are useful for inducing antibodies that react with the mimicked protein. Preferably, the amino acid sequence of the epitope-bearing peptide is selected to provide substantial solubility in aqueous solvents (*i.e.*, the sequence includes relatively hydrophilic residues and highly hydrophobic sequences are preferably avoided); sequences containing proline residues are particularly preferred.

15        Non-limiting examples of epitope-bearing polypeptides or peptides that can be used to generate antibodies specific for the polypeptides of the invention include certain epitope-bearing regions of the polypeptides comprising amino acid sequences encoded by polynucleotides comprising one or more of the recombination site-encoding nucleic acid molecules of the invention, including those encoding *attB1*, *attB2*, *attP1*, *attP2*, *attL1*, *attL2*, *attR1* and *attR2* having the nucleotide sequences set forth in Figure 9 (or a nucleotide sequence complementary thereto); the complete amino acid sequences encoded by the three reading frames of the polynucleotides contained in the deposited clones described herein; and the amino acid sequences encoded by all reading frames of polynucleotides which hybridize under stringent hybridization conditions to polynucleotides having the nucleotide sequences encoding the recombination site sequences (or portions thereof) of the invention as set forth in Figure 9 (or a nucleic acid sequence complementary thereto). Other epitope-bearing polypeptides or peptides that may be used to generate antibodies specific for the polypeptides

of the invention will be apparent to one of ordinary skill in the art based on the primary amino acid sequences of the polypeptides of the invention described herein, via the construction of Kyte-Doolittle hydrophilicity and Jameson-Wolf antigenic index plots of the polypeptides of the invention using, for example, PROTEAN computer software (DNASTAR, Inc.; Madison, Wisconsin).

The epitope-bearing peptides and polypeptides of the invention may be produced by any conventional means for making peptides or polypeptides including recombinant means using nucleic acid molecules of the invention. For instance, a short epitope-bearing amino acid sequence may be fused to a larger polypeptide which acts as a carrier during recombinant production and purification, as well as during immunization to produce anti-peptide antibodies. Epitope-bearing peptides also may be synthesized using known methods of chemical synthesis (*see, e.g.*, U.S. Patent No. 4,631,211 and Houghten, R. A., *Proc. Natl. Acad. Sci. USA* 82:5131-5135 (1985), both of which are incorporated by reference herein in their entireties).

As one of skill in the art will appreciate, the polypeptides of the present invention and epitope-bearing fragments thereof may be immobilized onto a solid support, by techniques that are well-known and routine in the art. By "solid support" is intended any solid support to which a peptide can be immobilized. Such solid supports include, but are not limited to nitrocellulose, diazocellulose, glass, polystyrene, polyvinylchloride, polypropylene, polyethylene, dextran, Sepharose, agar, starch, nylon, beads and microtitre plates. Linkage of the peptide of the invention to a solid support can be accomplished by attaching one or both ends of the peptide to the support. Attachment may also be made at one or more internal sites in the peptide. Multiple attachments (both internal and at the ends of the peptide) may also be used according to the invention. Attachment can be via an amino acid linkage group such as a primary amino group, a carboxyl group, or a sulfhydryl (SH) group or by chemical linkage groups such as with cyanogen bromide (CNBr) linkage through a spacer. For non-covalent attachments to the support, addition of an affinity tag sequence to the peptide can be used such as GST (Smith, D.B., and Johnson, K.S., *Gene* 67:31 (1988)), polyhistidines (Hochuli, E., *et al.*, *J. Chromatog.* 411:77 (1987)), or biotin. Such affinity tags

may be used for the reversible attachment of the peptide to the support. Such immobilized polypeptides or fragments may be useful, for example, in isolating antibodies directed against one or more of the polypeptides of the invention, or other proteins or peptides that recognize other proteins or peptides that bind to one or more of the polypeptides of the invention, as described below.

As one of skill in the art will also appreciate, the polypeptides of the present invention and the epitope-bearing fragments thereof described herein can be combined with one or more fusion partner proteins or peptides, or portions thereof, including but not limited to GST, His<sub>6</sub>, Trx, and portions of the constant domain of immunoglobulins (Ig), resulting in chimeric or fusion polypeptides. These fusion polypeptides facilitate purification of the polypeptides of the invention (EP 0 394 827; Traunecker *et al.*, *Nature* 331:84-86 (1988)) for use in analytical or diagnostic (including high-throughput) format.

### ***Antibodies***

In another aspect, the invention relates to antibodies that recognize and bind to the polypeptides (or epitope-bearing fragments thereof) or nucleic acid molecules (or portions thereof) of the invention. In a related aspect, the invention relates to antibodies that recognize and bind to one or more polypeptides encoded by all reading frames of one or more recombination site nucleic acid sequences or portions thereof, or to one or more nucleic acid molecules comprising one or more recombination site nucleic acid sequences or portions thereof, including but not limited to *att* sites (including *attB1*, *attB2*, *attP1*, *attP2*, *attL1*, *attL2*, *attR1*, *attR2* and the like), *lox* sites (*e.g.*, *loxP*, *loxP511*, and the like), FRT, and the like, or mutants, fragments, variants and derivatives thereof. See generally U.S. Patent No. 5,888,732, which is incorporated herein by reference in its entirety. The antibodies of the present invention may be polyclonal or monoclonal, and may be prepared by any of a variety of methods and in a variety of species according to methods that are well-known in the art. See, for instance, U.S. Patent No. 5,587,287; Sutcliffe, J.G., *et al.*, *Science* 219:660-666 (1983); Wilson *et al.*, *Cell* 37: 767 (1984); and Bittle, F.J., *et al.*, *J. Gen. Virol.* 66:2347-2354 (1985). Antibodies specific for any of the polypeptides or nucleic acid molecules described

herein, such as antibodies specifically binding to one or more of the polypeptides encoded by the recombination site nucleotide sequences, or one or more nucleic acid molecules, described herein or contained in the deposited clones, antibodies against fusion polypeptides (e.g., binding to fusion polypeptides between one or more of the fusion partner proteins and one or more of the recombination site polypeptides of the invention, as described herein), and the like, can be raised against the intact polypeptides or polynucleotides of the invention or one or more antigenic polypeptide fragments thereof.

As used herein, the term "antibody" (Ab) may be used interchangeably with the terms "polyclonal antibody" or "monoclonal antibody" (mAb), except in specific contexts as described below. These terms, as used herein, are meant to include intact molecules as well as antibody fragments (such as, for example, Fab and F(ab')<sub>2</sub> fragments) which are capable of specifically binding to a polypeptide or nucleic acid molecule of the invention or a portion thereof. It will therefore be appreciated that, in addition to the intact antibodies of the invention, Fab, F(ab')<sub>2</sub> and other fragments of the antibodies described herein, and other peptides and peptide fragments that bind one or more polypeptides or polynucleotides of the invention, are also encompassed within the scope of the invention. Such antibody fragments are typically produced by proteolytic cleavage of intact antibodies, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')<sub>2</sub> fragments). Antibody fragments, and peptides or peptide fragments, may also be produced through the application of recombinant DNA technology or through synthetic chemistry.

Epitope-bearing peptides and polypeptides, and nucleic acid molecules or portions thereof, of the invention may be used to induce antibodies according to methods well known in the art, as generally described herein (*see, e.g., Sutcliffe, et al., supra; Wilson, et al., supra; and Bittle, F. J., et al., J. Gen. Virol. 66:2347-2354 (1985)*).

Polyclonal antibodies according to this aspect of the invention may be made by immunizing an animal with one or more of the polypeptides or nucleic acid molecules of the invention described herein or portions thereof according to standard techniques (*see, e.g., Harlow, E., and Lane, D., Antibodies: A*

*Laboratory Manual*, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press (1988); Kaufman, P.B., *et al.*, In: *Handbook of Molecular and Cellular Methods in Biology and Medicine*, Boca Raton, Florida: CRC Press, pp. 468-469 (1995)). For producing antibodies that recognize and bind to the polypeptides or nucleic acid molecules of the invention or portions thereof, animals may be immunized with free peptide or free nucleic acid molecules; however, antibody titer may be boosted by coupling of the peptide to a macromolecular carrier, such as albumin, KLH, or tetanus toxoid (particularly for producing antibodies against the nucleic acid molecules of the invention or portions thereof; *see* Harlow and Lane, *supra*, at page 154), or to a solid phase carrier such as a latex or glass microbead. For instance, peptides containing cysteine may be coupled to carrier using a linker such as m-maleimidobenzoyl-N- hydroxysuccinimide ester (MBS), while other peptides may be coupled to carrier using a more general linking agent such as glutaraldehyde. Animals such as rabbits, rats and mice may be immunized with either free (if the polypeptide immunogen is larger than about 25 amino acids in length) or carrier-coupled peptides or nucleic acid molecules, for instance, by intraperitoneal and/or intradermal injection of emulsions containing about 100 µg peptide, polynucleotide, or carrier protein, and Freund's adjuvant. Several booster injections may be needed, for instance, at intervals of about two weeks, to provide a useful titer of antibody which can be detected, for example, by ELISA assay using free peptide or nucleic acid molecule adsorbed to a solid surface. In another approach, cells expressing one or more of the polypeptides or polynucleotides of the invention or an antigenic fragment thereof can be administered to an animal in order to induce the production of sera containing polyclonal antibodies, according to routine immunological methods. In yet another method, a preparation of one or more of the polypeptides or polynucleotides of the invention is prepared and purified as described herein, to render it substantially free of natural contaminants. Such a preparation may then be introduced into an animal in order to produce polyclonal antisera of greater specific activity. The titer of antibodies in serum from an immunized animal, regardless of the method of immunization used, may be increased by selection of anti-peptide or anti-polynucleotide antibodies, for



instance, by adsorption to the peptide or polynucleotide on a solid support and elution of the selected antibodies according to methods well known in the art.

In an alternative method, the antibodies of the present invention are monoclonal antibodies (or fragments thereof which bind to one or more of the polypeptides of the invention). Such monoclonal antibodies can be prepared using hybridoma technology (Kohler *et al.*, *Nature* 256:495 (1975); Köhler *et al.*, *Eur. J. Immunol.* 6:511 (1976); Köhler *et al.*, *Eur. J. Immunol.* 6:292 (1976); Hammerling *et al.*, In: *Monoclonal Antibodies and T-Cell Hybridomas*, Elsevier, N.Y., pp. 563-681 (1981)). In general, such procedures involve immunizing an animal (preferably a mouse) with a polypeptide or polynucleotide of the invention (or a fragment thereof), or with a cell expressing a polypeptide or polynucleotide of the invention (or a fragment thereof). The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP<sub>2</sub>O), available from the American Type Culture Collection, Rockville, Maryland. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands *et al.* (*Gastroenterol.* 80:225-232 (1981)). The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding one or more of the polypeptides or nucleic acid molecules of the invention, or fragments thereof. Hence, the present invention also provides hybridoma cells and cell lines producing monoclonal antibodies of the invention, particularly that recognize and bind to one or more of the polypeptides or nucleic acid molecules of the invention.

Alternatively, additional antibodies capable of binding to one or more of the polypeptides of the invention, or fragments thereof, may be produced in a two-step procedure through the use of anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and that, therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, antibodies specific for one or more of the polypeptides or polynucleotides of the invention, prepared as described above, are used to immunize an animal, preferably a mouse. The splenocytes of such an

animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to an antibody specific for one or more of the polypeptides or polynucleotides of the invention can be blocked by polypeptides of the invention themselves. Such antibodies comprise anti-idiotypic antibodies to the antibodies recognizing one or more of the polypeptides or polynucleotides of the invention, and can be used to immunize an animal to induce formation of further antibodies specific for one or more of the polypeptides or polynucleotides of the invention.

For use, the antibodies of the invention may optionally be detectably labeled by covalent or non-covalent attachment of one or more labels, including but not limited to chromogenic, enzymatic, radioisotopic, isotopic, fluorescent, toxic, chemiluminescent, or nuclear magnetic resonance contrast agents or other labels.

Examples of suitable enzyme labels include malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast-alcohol dehydrogenase, alpha-glycerol phosphate dehydrogenase, triose phosphate isomerase, peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase, and acetylcholine esterase.

Examples of suitable radioisotopic labels include  $^3\text{H}$ ,  $^{111}\text{In}$ ,  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{32}\text{P}$ ,  $^{35}\text{S}$ ,  $^{14}\text{C}$ ,  $^{51}\text{Cr}$ ,  $^{57}\text{To}$ ,  $^{58}\text{Co}$ ,  $^{59}\text{Fe}$ ,  $^{75}\text{Se}$ ,  $^{152}\text{Eu}$ ,  $^{90}\text{Y}$ ,  $^{67}\text{Cu}$ ,  $^{217}\text{Ci}$ ,  $^{211}\text{At}$ ,  $^{212}\text{Pb}$ ,  $^{47}\text{Sc}$ ,  $^{109}\text{Pd}$ , etc.  $^{111}\text{In}$  is a preferred isotope where in vivo imaging is used since it avoids the problem of dehalogenation of the  $^{125}\text{I}$  or  $^{131}\text{I}$ -labeled monoclonal antibody by the liver. In addition, this radionucleotide has a more favorable gamma emission energy for imaging (Perkins *et al.*, *Eur. J. Nucl. Med.* 10:296-301 (1985); Carasquillo *et al.*, *J. Nucl. Med.* 28:281-287 (1987)). For example,  $^{111}\text{In}$  coupled to monoclonal antibodies with 1-(P-isothiocyanatobenzyl)-DPTA has shown little uptake in non-tumorous tissues, particularly the liver, and therefore enhances specificity of tumor localization (Esteban *et al.*, *J. Nucl. Med.* 28:861-870 (1987)).

Examples of suitable non-radioactive isotopic labels include  $^{157}\text{Gd}$ ,  $^{55}\text{Mn}$ ,  $^{162}\text{Dy}$ ,  $^{52}\text{Tr}$ , and  $^{56}\text{Fe}$ .

Examples of suitable fluorescent labels include an  $^{152}\text{Eu}$  label, a fluorescein label, an isothiocyanate label, a rhodamine label, a phycoerythrin label, a

phycocyanin label, an allophycocyanin label, an o-phthaldehyde label, a green fluorescent protein (GFP) label, and a fluorescamine label.

Examples of suitable toxin labels include diphtheria toxin, ricin, and cholera toxin.

5        Examples of chemiluminescent labels include a luminal label, an isoluminal label, an aromatic acridinium ester label, an imidazole label, an acridinium salt label, an oxalate ester label, a luciferin label, a luciferase label, and an aequorin label.

10       Examples of nuclear magnetic resonance contrasting agents include heavy metal nuclei such as Gd, Mn, and iron.

Typical techniques for binding the above-described labels to the antibodies of the invention are provided by Kennedy *et al.*, *Clin. Chim. Acta* 70:1-31 (1976), and Schurs *et al.*, *Clin. Chim. Acta* 81:1-40 (1977). Coupling techniques mentioned in the latter are the glutaraldehyde method, the periodate method, the dimaleimide method, the m-maleimidobenzyl-N-hydroxy-succinimide ester method, all of which methods are incorporated by reference herein.

15       It will be appreciated by one of ordinary skill that the antibodies of the present invention may alternatively be coupled to a solid support, to facilitate, for example, chromatographic and other immunological procedures using such solid phase-immobilized antibodies. Included among such procedures are the use of the antibodies of the invention to isolate or purify polypeptides comprising one or more epitopes encoded by the nucleic acid molecules of the invention (which may be fusion polypeptides or other polypeptides of the invention described herein), or to isolate or purify polynucleotides comprising one or more recombination site sequences of the invention or portions thereof. Methods for isolation and purification of polypeptides (and, by analogy, polynucleotides) by affinity chromatography, for example using the antibodies of the invention coupled to a solid phase support, are well-known in the art and will be familiar to one of ordinary skill. The antibodies of the invention may also be used in other applications, for example to cross-link or couple two or more proteins, polypeptides, polynucleotides, or portions thereof into a structural and/or functional complex. In one such use, an antibody of the invention may have two

20

25

30

or more distinct epitope-binding regions that may bind, for example, a first polypeptide (which may be a polypeptide of the invention) at one epitope-binding region on the antibody and a second polypeptide (which may be a polypeptide of the invention) at a second epitope-binding region on the antibody, thereby bringing the first and second polypeptides into close proximity to each other such that the first and second polypeptides are able to interact structurally and/or functionally (as, for example, linking an enzyme and its substrate to carry out enzymatic catalysis, or linking an effector molecule and its receptor to carry out or induce a specific binding of the effector molecule to the receptor or a response to the effector molecule mediated by the receptor). Additional applications for the antibodies of the invention include, for example, the preparation of large-scale arrays of the antibodies, polypeptides, or nucleic acid molecules of the invention, or portions thereof, on a solid support, for example to facilitate high-throughput screening of protein or RNA expression by host cells containing nucleic acid molecules of the invention (known in the art as "chip array" protocols; *see, e.g.*, U.S. Patent Nos. 5,856,101, 5,837,832, 5,770,456, 5,744,305, 5,631,734, and 5,593,839, which are directed to production and use of chip arrays of polypeptides (including antibodies) and polynucleotides, and the disclosures of which are incorporated herein by reference in their entireties). By "solid support" is intended any solid support to which an antibody can be immobilized. Such solid supports include, but are not limited to nitrocellulose, diazocellulose, glass, polystyrene, polyvinylchloride, polycarbonate, polypropylene, polyethylene, dextran, Sepharose, agar, starch, nylon, beads and microtitre plates. Preferred are beads made of glass, latex or a magnetic material. Linkage of an antibody of the invention to a solid support can be accomplished by attaching one or both ends of the antibody to the support. Attachment may also be made at one or more internal sites in the antibody. Multiple attachments (both internal and at the ends of the antibody) may also be used according to the invention. Attachment can be via an amino acid linkage group such as a primary amino group, a carboxyl group, or a sulfhydryl (SH) group or by chemical linkage groups such as with cyanogen bromide (CNBr) linkage through a spacer. For non-covalent attachments, addition of an affinity tag sequence to the peptide can be used such as GST

(Smith, D.B., and Johnson, K.S., *Gene* 67:31 (1988)), polyhistidines (Hochuli, E., *et al.*, *J. Chromatog.* 411:77 (1987)), or biotin. Alternatively, attachment can be accomplished using a ligand which binds the Fc region of the antibodies of the invention, *e.g.*, protein A or protein G. Such affinity tags may be used for the reversible attachment of the antibodies to the support. Peptides may also be recognized via specific ligand-receptor interactions or using phage display methodologies that will be familiar to the skilled artisan, for their ability to bind polypeptides of the invention or fragments thereof.

### **Kits**

In another aspect, the invention provides kits which may be used in producing the nucleic acid molecules, polypeptides, vectors, host cells, and antibodies, and in the recombinational cloning methods, of the invention. Kits according to this aspect of the invention may comprise one or more containers, which may contain one or more of the nucleic acid molecules, primers, polypeptides, vectors, host cells, or antibodies of the invention. In particular, a kit of the invention may comprise one or more components (or combinations thereof) selected from the group consisting of one or more recombination proteins (*e.g.*, Int) or auxiliary factors (*e.g.* IHF and/or Xis) or combinations thereof, one or more compositions comprising one or more recombination proteins or auxiliary factors or combinations thereof (for example, GATEWAY™ LR Clonase™ Enzyme Mix or GATEWAY™ BP Clonase™ Enzyme Mix) one or more Destination Vector molecules (including those described herein), one or more Entry Clone or Entry Vector molecules (including those described herein), one or more primer nucleic acid molecules (particularly those described herein), one or more host cells (*e.g.* competent cells, such as *E. coli* cells, yeast cells, animal cells (including mammalian cells, insect cells, nematode cells, avian cells, fish cells, etc.), plant cells, and most particularly *E. coli* DB3, DB3.1 (preferably *E. coli* LIBRARY EFFICIENCY® DB3.1™ Competent Cells; Life Technologies, Inc., Rockville, MD), DB4 and DB5; *see* U.S. Provisional Application No. 60/122,392, filed on March 2, 1999, and the corresponding U.S. Utility Application No. \_\_\_\_\_ of Hartley *et al.*, entitled "Cells Resistant to Toxic Genes and Uses Thereof," filed

on even day herewith, the disclosures of which are incorporated by reference herein in its entirety), and the like. In related aspects, the kits of the invention may comprise one or more nucleic acid molecules encoding one or more recombination sites or portions thereof, such as one or more nucleic acid molecules comprising  
5 a nucleotide sequence encoding the one or more recombination sites (or portions thereof) of the invention, and particularly one or more of the nucleic acid molecules contained in the deposited clones described herein. Kits according to this aspect of the invention may also comprise one or more isolated nucleic acid molecules of the invention, one or more vectors of the invention, one or more  
10 primer nucleic acid molecules of the invention, and/or one or more antibodies of the invention. The kits of the invention may further comprise one or more additional containers containing one or more additional components useful in combination with the nucleic acid molecules, polypeptides, vectors, host cells, or antibodies of the invention, such as one or more buffers, one or more detergents,  
15 one or more polypeptides having nucleic acid polymerase activity, one or more polypeptides having reverse transcriptase activity, one or more transfection reagents, one or more nucleotides, and the like. Such kits may be used in any process advantageously using the nucleic acid molecules, primers, vectors, host cells, polypeptides, antibodies and other compositions of the invention, for  
20 example in methods of synthesizing nucleic acid molecules (*e.g.*, via amplification such as via PCR), in methods of cloning nucleic acid molecules (preferably via recombinational cloning as described herein), and the like.

### ***Optimization of Recombinational Cloning System***

25 The usefulness of a particular nucleic acid molecule, or vector comprising a nucleic acid molecule, of the invention in methods of recombinational cloning may be determined by any one of a number of assay methods. For example, Entry and Destination vectors of the present invention may be assessed for their ability to function (*i.e.*, to mediate the transfer of a nucleic acid molecule, DNA segment,  
30 gene, cDNA molecule or library from a cloning vector to an Expression Vector) by carrying out a recombinational cloning reaction as described in more detail in the Examples below and as described in U.S. Application Nos. 08/663,002, filed

June 7, 1996 (now U.S. Patent No. 5,888,732), 09/005,476, filed January 12, 1998, 09/177,387, filed October 23, 1998, and 60/108,324, filed November 13, 1998, the disclosures of which are incorporated by reference herein in their entireties. Alternatively, the functionality of Entry and Destination Vectors prepared according to the invention may be assessed by examining the ability of these vectors to recombine and create cointegrate molecules, or to transfer a nucleic acid molecule of interest, using an assay such as that described in detail below in Example 19. Analogously, the formulation of compositions comprising one or more recombination proteins or combinations thereof, for example GATEWAY™ LR Clonase™ Enzyme Mix and GATEWAY™ BP Clonase™ Enzyme Mix, may be optimized using assays such as those described below in Example 18.

### *Uses*

There are a number of applications for the compositions, methods and kits of the present invention. These uses include, but are not limited to, changing vectors, targeting gene products to intracellular locations, cleaving fusion tags from desired proteins, operably linking nucleic acid molecules of interest to regulatory genetic sequences (*e.g.*, promoters, enhancers, and the like), constructing genes for fusion proteins, changing copy number, changing replicons, cloning into phages, and cloning, *e.g.*, PCR products, genomic DNAs, and cDNAs. In addition, the nucleic acid molecules, vectors, and host cells of the invention may be used in the production of polypeptides encoded by the nucleic acid molecules, in the production of antibodies directed against such polypeptides, in recombinational cloning of desired nucleic acid sequences, and in other applications that may be enhanced or facilitated by the use of the nucleic acid molecules, vectors, and host cells of the invention.

In particular, the nucleic acid molecules, vectors, host cells, polypeptides, antibodies, and kits of the invention may be used in methods of transferring one or more desired nucleic acid molecules or DNA segments, for example one or more genes, cDNA molecules or cDNA libraries, into a cloning or Expression Vector for use in transforming additional host cells for use in cloning or

amplification of, or expression of the polypeptide encoded by, the desired nucleic acid molecule or DNA segment. Such recombinational cloning methods which may advantageously use the nucleic acid molecules, vectors, and host cells of the invention, are described in detail in the Examples below, and in commonly owned  
5 U.S. Application Nos. 08/486,139, filed June 7, 1995, 08/663,002, filed June 7, 1996 (now U.S. Patent No. 5,888,732), 09/005,476, filed January 12, 1998, 09/177,387, filed October 23, 1998, and 60/108,324, filed November 13, 1998, the disclosures of all of which are incorporated by reference herein in their entireties.

10 It will be understood by one of ordinary skill in the relevant arts that other suitable modifications and adaptations to the methods and applications described herein are readily apparent from the description of the invention contained herein in view of information known to the ordinarily skilled artisan, and may be made  
15 without departing from the scope of the invention or any embodiment thereof. Having now described the present invention in detail, the same will be more clearly understood by reference to the following examples, which are included herewith for purposes of illustration only and are not intended to be limiting of the invention.

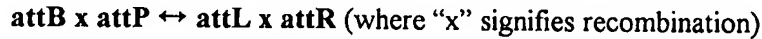
## 20 *Examples*

### *Example 1: Recombination Reactions of Bacteriophage $\lambda$*

25 The *E. coli* bacteriophage  $\lambda$  can grow as a lytic phage, in which case the host cell is lysed, with the release of progeny virus. Alternatively, lambda can integrate into the genome of its host by a process called lysogenization (see Figure 60). In this lysogenic state, the phage genome can be transmitted to daughter cells for many generations, until conditions arise that trigger its excision from the genome.  
30 At this point, the virus enters the lytic part of its life cycle. The control of the switch between the lytic and lysogenic pathways is one of the best understood processes in molecular biology (M. Ptashne, *A Genetic Switch*, Cell Press, 1992).



The integrative and excisive recombination reactions of  $\lambda$ , performed *in vitro*, are the basis of Recombinational Cloning System of the present invention. They can be represented schematically as follows:



10                    The four att sites contain binding sites for the proteins that mediate the reactions. The wild type attP, attB, attL, and attR sites contain about 243, 25, 100, and 168 base pairs, respectively. The attB x attP reaction (hereinafter referred to as a "BP Reaction," or alternatively and equivalently as an "Entry Reaction" or a "Gateward Reaction") is mediated by the proteins Int and IHF. The attL x attR reaction (hereinafter referred to as an "LR Reaction," or alternatively and equivalently as a "Destination Reaction") is mediated by the proteins Int, IHF, and Xis. Int (integrase) and Xis (excisionase) are encoded by the  $\lambda$  genome, while IHF (integration host factor) is an *E. coli* protein. For a general review of lambda recombination, see: A. Landy, *Ann. Rev. Biochem.* 58: 913-949 (1989).

20                    ***Example 2: Recombination Reactions of the Recombinational Cloning System***

The LR Reaction -- the exchange of a DNA segment from an Entry Clone to a Destination Vector -- is the *in vitro* version of the  $\lambda$  excision reaction:



30                    There is a practical imperative for this configuration: after an LR Reaction in one configuration of the present method, an att site usually separates a functional motif (such as a promoter or a fusion tag) from a nucleic acid molecule of interest in an Expression Clone, and the 25 bp attB site is much smaller than the attP, attL, and attR sites.

Note that the recombination reaction is conservative, i.e., there is no net synthesis or loss of base pairs. The DNA segments that flank the recombination

sites are merely switched. The wild type  $\lambda$  recombination sites are modified for purposes of the GATEWAY™ Cloning System, as follows:

To create certain preferred Destination Vectors, a part (43 bp) of attR was removed, to make the excise reaction irreversible and more efficient (W. Bushman et al., *Science* 230: 906, 1985). The attR sites in preferred Destination Vectors of the invention are 125 bp in length. Mutations were made to the core regions of the att sites, for two reasons: (1) to eliminate stop codons, and (2) to ensure specificity of the recombination reactions (i.e., attR1 reacts only with attL1, attR2 reacts only with attL2, etc.).

Other mutations were introduced into the short (5 bp) regions flanking the 15 bp core regions of the attB sites to minimize secondary structure formation in single-stranded forms of attB plasmids, e.g., in phagemid ssDNA or in mRNA. Sequences of attB1 and attB2 to the left and right of a nucleic acid molecule of interest after it has been cloned into a Destination Vector are given in Figure 6.

Figure 61 illustrates how an Entry Clone and a Destination Vector recombine in the LR Reaction to form a co-integrate, which resolves through a second reaction into two daughter molecules. The two daughter molecules have the same general structure regardless of which pair of sites, attL1 and attR1 or attL2 and attR2, react first to form the co-integrate. The segments change partners by these reactions, regardless of whether the parental molecules are both circular, one is circular and one is linear, or both are linear. In this example, selection for ampicillin resistance carried on the Destination Vector, which also carries the death gene *ccdB*, provides the means for selecting only for the desired attB product plasmid.

### **Example 3: Protein Expression in the Recombinational Cloning System**

Proteins are expressed *in vivo* as a result of two processes, transcription (DNA into RNA), and translation (RNA into protein). For a review of protein expression in prokaryotes and eukaryotes, see Example 13 below. Many vectors (pUC, BlueScript, pGem) use interruption of a transcribed *lacZ* gene for blue-white screening. These plasmids, and many Expression Vectors, use the *lac* promoter to control expression of cloned genes. Transcription from the *lac*

promoter is turned on by adding the inducer IPTG. However, a low level of RNA is made in the absence of inducer, i.e., the lac promoter is never completely off. The result of this "leakiness" is that genes whose expression is harmful to *E. coli* may prove difficult or impossible to clone in vectors that contain the lac promoter, or they may be cloned only as inactive mutants.

In contrast to other gene expression systems, nucleic acid molecules cloned into an Entry Vector may be designed *not* to be expressed. The presence of the strong transcriptional terminator *rrnB* (Orosz, et al., *Eur. J. Biochem.* 201: 653, 1991) just upstream of the attL1 site keeps transcription from the vector promoters (drug resistance and replication origin) from reaching the cloned gene. However, if a toxic gene is cloned into a Destination Vector, the host may be sick, just as in other expression systems. But the reliability of subcloning by *in vitro* recombination makes it easier to recognize that this has happened -- and easier to try another expression option in accordance with the methods of the invention, if necessary.

#### **Example 4: Choosing the Right Entry Vector**

There are two kinds of choices that must be made in choosing the best Entry Vector, dictated by (1) the particular DNA segment that is to be cloned, and (2) what is to be accomplished with the cloned DNA segment. These factors are critical in the choice of Entry Vector used, because when the desired nucleic acid molecule of interest is moved from the Entry Vector to a Destination Vector, all the base pairs between the nucleic acid molecule of interest and the Int cutting sites in attL1 and attL2 (such as in Figure 6) move into the Destination Vector as well. For genomic DNAs that are not expressed as a result of moving into a Destination Vector, these decisions are not as critical.

For example, if an Entry Vector with certain translation start signals is used, those sequences will be translated into amino acids if an amino-terminal fusion to the desired nucleic acid molecule of interest is made. Whether the desired nucleic acid molecule of interest is to be expressed as fusion protein, native protein, or both, dictates whether translational start sequences must be included between the attB sites of the clone (native protein) or, alternatively, supplied by the Destination

Vector (fusion protein). In particular, Entry Clones that include translational start sequences may prove less suitable for making fusion proteins, as internal initiation of translation at these sites can decrease the yield of N-terminal fusion protein. These two types of expression afforded by the compositions and methods of the invention are illustrated in Figure 62.

No Entry Vector is likely to be optimal for all applications. The nucleic acid molecule of interest may be cloned into any of several optimal Entry Vectors.

As an example, consider pENTR7 (Figure 16) and pENTR11 (Figure 20), which are useful in a variety of applications, including (but not limited to):

- Cloning cDNAs from most of the commercially available libraries. The sites to the left and right of the *ccdB* death gene have been chosen so that directional cloning is possible if the DNA to be cloned does not have two or more of these restriction sites.

- Cloning of genes directionally: *Sall*, *Bam*HI, *Xmn*I (blunt), or *Kpn*I on the left of *ccdB*; *Not*I, *Xho*I, *Xba*I, or *Eco*RV (blunt), on the right.

- Cloning of genes or gene fragments with a blunt amino end at the *Xmn*I site. The *Xmn*I site has four of the six most favored bases for eukaryotic expression (see Example 13, below), so that if the first three bases of the DNA to be cloned are ATG, the open reading frame (ORF) will be expressed in eukaryotic cells (e.g., mammalian cells, insect cells, yeast cells) when it is transcribed in the appropriate Destination Vector. In addition, in pENTR11, a Shine-Dalgarno sequence is situated 8 bp upstream, for initiating protein synthesis in a prokaryotic host cell (particularly a bacterial cell, such as *E. coli*) at an ATG.

- Cleaving off amino terminal fusions (e.g., His<sub>6</sub>, GST, or thioredoxin) using the highly specific TEV (Tobacco Etch Virus) protease (available from Life Technologies, Inc.). If the nucleic acid molecule of interest is cloned at the

blunt *Xmn*I site, TEV cleavage will leave two amino acids on the amino end of the expressed protein.

•Selecting against uncut or singly cut Entry Vector molecules during cloning with restriction enzymes and ligase. If the *ccdB* gene is not removed with a double digest, it will kill any recipient *E. coli* cell that does not contain a mutation that makes the cell resistant to *ccdB* (see U.S. Provisional Application No. 60/122,392, filed on March 2, 1999, the disclosure of which is incorporated by reference herein in its entirety).

•Allowing production of amino fusions with ORFs in all cloning sites. There are no stop codons (in the attL1 reading frame) upstream of the *ccdB* gene.

In addition, pENTR11 is also useful in the following applications:

•Cloning cDNAs that have an *Nco*I site at the initiating ATG into the *Nco*I site. Similar to the *Xmn*I site, this site has four of the six most favored bases for eukaryotic expression. Also, a Shine-Dalgarno sequence is situated 8 bp upstream, for initiating protein synthesis in a prokaryotic host cell (particularly a bacterial cell, such as *E. coli*) at an ATG.

•Producing carboxy fusion proteins with ORFs positioned in phase with the reading frame convention for carboxy-terminal fusions (see Figure 20A).

Table 1 lists some non-limiting examples of Entry Vectors and their characteristics, and Figures 10-20 show their cloning sites. All of the Entry Vectors listed in Table 1 are available commercially from Life Technologies, Inc., Rockville, Maryland. Other Entry Vectors not specifically listed here, which comprise alternative or additional features may be made by one of ordinary skill using routine methods of molecular and cellular biology, in view of the disclosure contained herein.

Table 1 Examples of Entry Vectors

Designation	Mnemonic Name	Class of Entry Vector	Distinctive Cloning Sites	Amino Fusions	Native Protein in E.coli	Native Protein in Eukaryotic Cells	Protein Synthesis Features
pENTR-1A, 2B, 3C	Minimal blunt RF A, B, C	Alternative Reading Frame Vectors	Reading frame A, B, or C; blunt cut closest to attL1	Good	Poor	Good	Minimal amino acids between tag and protein; no SD
pENTR4	Minimal Nco	Restr. Enz. Cleavage Vectors	Nco I site (common in euk. cDNAs) closest to attL1	Good	Poor	Good	Good Kozac; no SD
pENTR5	Minimal Nde	Restr. Enz. Cleavage Vectors	NdeI site closest to attL1	Good	Poor	Poor at Nde I, Good at Xmn I	No SD; poor Kozac at Nde, good at Xmn
pENTR6	Minimal Sph	Restr. Enz. Cleavage Vectors	Sph I site closest to attL1	Good	Poor	Poor at Sph I, Good at Xmn I	No SD; poor Kozac at Sph, good at Xmn
pENTR7	TEV Blunt	TEV Cleavage Site Present	Xmn I (blunt) is first cloning site after TEV site	Good	Poor	Good at Xmn I site	TEV protease leaves Gly-Thr on amino end of protein; no SD
pENTR8	TEV Nco	TEV Cleavage Site Present	Nco I is first cloning site after TEV site	Good	Poor	Good	TEV protease leaves Gly-Thr on amino end of protein; no SD

pENTR9	TEV Nde	TEV Cleavage Site Present	Nde I is first cloning site after TEV site	Good	Poor	Poor	TEV protease leaves Gly-Thr on amino end of protein; no SD, poor Kozac
pENTR10	Nde with SD	Good SD for E.coli Expression	Strong SD; Nde I site, no TEV	Poor	Good	Poor	Strong SD, internal starts in amino fusions. Poor Kz. No TEV
pENTR11	2 X SD+Kozac	Good SD for E.coli Expression	Xmn I (blunt) and Nco I sites each preceded by SD and Kozac	Good	Good	Good	Strong SD/Koz Internal starts in amino fusions. No TEV

Entry vectors pENTR1A (Figures 10A and 10B), pENTR2B (Figures 11A and 11B), and pENTR3C (Figures 12A and 12B) are almost identical, except that the restriction sites are in different reading frames. Entry vectors pENTR4 (Figures 13A and 13B), pENTR5 (Figures 14A and 14B), and pENTR6 (Figures 15A and 15B) are essentially identical to pENTR1A, except that the blunt *DraI* site has been replaced with sites containing the ATG methionine codon: *NcoI* in pENTR4, *NdeI* in pENTR5, and *SphI* in pENTR6. Nucleic acid molecules that contain one of these sites at the initiating ATG can be conveniently cloned in these Entry vectors. The *NcoI* site in pENTR4 is especially useful for expression of nucleic acid molecules in eukaryotic cells, since it contains many of the bases that give efficient translation (*see* Example 13, below). (Nucleic acid molecules of interest cloned into the *NdeI* site of pENTR5 are not expected to be highly expressed in eukaryotic cells, because the cytosine at position -3 from the initiating ATG is rare in eukaryotic genes.)

Entry vectors pENTR7 (Figures 16A and 16B), pENTR8 (Figures 17A and 17B), and pENTR9 (Figures 18A and 18B) contain the recognition site for the TEV protease between the attL1 site and the cloning sites. Cleavage sites for *XmnI* (blunt), *NcoI*, and *NdeI*, respectively, are the most 5' sites in these Entry vectors. Amino fusions can be removed efficiently if nucleic acid molecules are cloned into these Entry vectors. TEV protease is highly active and highly specific.

#### ***Example 5: Controlling Reading Frame***

One of the trickiest tasks in expression of cloned nucleic acid molecules is making sure the reading frame is correct. (Reading frame is important if fusions are being made between two ORFs, for example between a nucleic acid molecule of interest and a His6 or GST domain.) For purposes of the present invention, the following convention has been adopted: The reading frame of the DNA cloned into any Entry Vector must be in phase with that of the attB1 site shown in Figure 16A, pENTR7. Notice that the six As of the attL1 site are split into two lysine codons (aaa aaa). The Destination Vectors that make amino fusions were constructed such that they enter the attR1 site in this reading frame.



Destination Vectors for carboxy terminal fusions were also constructed, including those containing His<sub>6</sub> (pDEST23; Figure 43), GST (pDEST24; Figure 44), or thioredoxin (pDEST25; Figure 45) C-terminal fusion sequences.

Therefore, if a nucleic acid molecule of interest is cloned into an Entry Vector so that the aaa aaa reading frame within the attL1 site is in phase with the nucleic acid molecule's ORF, amino terminal fusions will automatically be correctly phased, for all the fusion tags. This is a significant improvement over the usual case, where each different vector can have different restriction sites and different reading frames.

See Example 15 for a practical example of how to choose the most appropriate combinations of Entry Vector and Destination Vector.

### Materials

Unless otherwise indicated, the following materials were used in the remaining Examples included herein:

#### 5X LR Reaction Buffer:

200-250 mM (preferably 250 mM) Tris-HCl, pH 7.5

250-350 mM (preferably 320 mM) NaCl

1.25-5 mM (preferably 4.75 mM) EDTA

12.5-35 mM (preferably 22-35 mM, and most preferably 35 mM)

Spermidine-HCl

1 mg/ml bovine serum albumin

#### GATEWAY™ LR Clonase™ Enzyme Mix:

per 4 µl of 1X LR Reaction Buffer:

150 ng carboxy-His6-tagged Int (see U.S. Appl. Nos. 60/108,324, filed November 13, 1998, and 09/438,358, filed November 12, 1999, both entirely incorporated by reference herein)

-106-

25 ng carboxy-His6-tagged Xis (see U.S. Appl. Nos. 60/108,324, filed  
November 13, 1998, and 09/438,358, filed November 12,  
1999, both entirely incorporated by reference herein)

30 ng IHF

50% glycerol

**5X BP Reaction Buffer:**

125 mM Tris-HCl, pH 7.5

110 mM NaCl

25 mM EDTA

25 mM Spermidine-HCl

5 mg/ml bovine serum albumin

**GATEWAY™ BP Clonase™ Enzyme Mix:**

per 4 µl of 1X BP Reaction Buffer:

200 ng carboxy-His6-tagged Int (see U.S. Appl. Nos. 60/108,324, filed  
November 13, 1998, and 09/438,358, filed November 12,  
1999, both entirely incorporated by reference herein)

80 ng IHF

50% glycerol

**10X Clonase Stop Solution:**

50 mM Tris-HCl, pH 8.0

1 mM EDTA

2 mg/ml Proteinase K

***Example 6: LR ("Destination") Reaction***

To create a new Expression Clone containing the nucleic acid molecule of  
interest (and which may be introduced into a host cell, ultimately for production  
of the polypeptide encoded by the nucleic acid molecule), an Entry Clone or  
Vector containing the nucleic acid molecule of interest, prepared as described

herein, is reacted with a Destination Vector. In the present example, a  $\beta$ -Gal gene flanked by attL sites is transferred from an Entry Clone to a Destination Vector.

Materials needed:

- 5       • 5 X LR Reaction buffer
- Destination Vector (preferably linearized), 75-150 ng/ $\mu$ l
- Entry Clone containing nucleic acid molecule of interest, 100-300 ng in  $\leq 8 \mu$ l TE buffer
- Positive control Entry Clone (pENTR- $\beta$ -Gal) DNA (See note, below)
- 10      • Positive control Destination Vector, pDEST1 (pTrc), 75 ng/ $\mu$ l
- GATEWAY™ LR Clonase™ Enzyme Mix (stored at - 80° C)
- 10X Clonase Stop solution
- pUC19 DNA, 10 pg/ $\mu$ l
- Chemically competent *E. coli* cells (competence:  $\geq 1 \times 10^7$  CFU/ $\mu$ g), 400  $\mu$ l.
- 15      • LB Plates containing ampicillin (100  $\mu$ g/ml) and methicillin (200  $\mu$ g/ml)  $\pm$  X-gal and IPTG (See below)

Notes:

20       Preparation of the Entry Clone DNA: Miniprep DNA that has been treated with RNase works well. A reasonably accurate quantitation ( $\pm 50\%$ ) of the DNA to be cloned is advised, as the GATEWAY™ reaction appears to have an optimum of about 100-300 ng of Entry Clone per 20  $\mu$ l of reaction mix.

25       The positive control Entry Clone, pENTR- $\beta$ -Gal, permits functional analysis of clones based on the numbers of expected blue vs. white colonies on LB plates containing IPTG + Bluo-gal (or X-gal), in addition to ampicillin (100  $\mu$ g/ml) and methicillin (200  $\mu$ g/ml). Because  $\beta$ -Galactosidase is a large protein, it often yields a less prominent band than many smaller proteins do on SDS protein gels.

30       In the Positive Control Entry Vector pENTR- $\beta$ -Gal, the coding sequence of  $\beta$ -Gal has been cloned into pENTR11 (Figures 20A and 20B), with translational start signals permitting expression in *E. coli*, as well as in eukaryotic

cells. The positive control Destination Vector, for example pDEST1 (Figure 21), is preferably linearized.

To prepare X-gal + IPTG plates, either of the following protocols may be used:

5

A. With a glass rod, spread over the surface of an LB agar plate: 40  $\mu$ l of 20 mg/ml X-gal (or Bluo-gal) in DMF plus 4  $\mu$ l 200 mg/ml IPTG. Allow liquid to adsorb into agar for 3-4 hours at 37° C before plating cells.

10

B. To liquid LB agar at ~45°C, add: X-gal (or Bluo-Gal) (20 mg/ml in DMF) to make 50  $\mu$ g/ml and IPTG (200 mM in water) to make 0.5-1 mM, just prior to pouring plates. Store X-gal and Bluo-Gal in a light-shielded container.

15

Colony color may be enhanced by placing the plates at 5° C for a few hours after the overnight incubation at 37°C. Protocol B can give more consistent colony color than A, but A is more convenient when selection plates are needed on short notice.

20

Recombination in Clonase reactions continues for many hours. While incubations of 45-60 minutes are usually sufficient, reactions with large DNAs, or in which both parental DNAs are supercoiled, or which will be transformed into cells of low competence, can be improved with longer incubation times, such as 2-24 hours at 25°C.

#### Procedure:

25

1. Assemble reactions as follows (combine all components at room temperature, except GATEWAY™ LR Clonase™ Enzyme Mix ("Clonase LR"), before removing Clonase LR from frozen storage):

Component	Tube 1	Tube 2	Tube 3	Tube 4
	Neg.	Pos.	Neg.	Test
p-Gate- $\beta$ Gal, (Positive control Entry Clone) 75 ng/ $\mu$ l	4 $\mu$ l	4 $\mu$ l		
pDEST1 (Positive control Destination Vector), 75 ng/ $\mu$ l	4 $\mu$ l	4 $\mu$ l		
Your Entry Clone (100-300 ng)			1 - 8 $\mu$ l	1 - 8 $\mu$ l
Destination Vector for your nucleic acid molecule, 75 ng/ $\mu$ l			4 $\mu$ l	4 $\mu$ l
5 X LR Reaction Buffer	4 $\mu$ l	4 $\mu$ l	4 $\mu$ l	4 $\mu$ l
TE	8 $\mu$ l	4 $\mu$ l	To 20 $\mu$ l	To 16 $\mu$ l
GATEWAY™ LR Clonase™ Enzyme Mix (store at - 80° C, add last)	---	4 $\mu$ l	---	4 $\mu$ l
Total Volume	20 $\mu$ l	20 $\mu$ l	20 $\mu$ l	20 $\mu$ l

2. Remove the GATEWAY™ LR Clonase™ Enzyme Mix from the -80° C freezer, place immediately on ice. The Clonase takes only a few minutes to thaw.
3. Add 4  $\mu$ l of GATEWAY™ LR Clonase™ Enzyme Mix to reactions #2 and #4;
4. Return GATEWAY™ LR Clonase™ Enzyme Mix to - 80° C freezer.
5. Incubate tubes at 25° for at least 60 minutes.
6. Add 2  $\mu$ l Clonase Stop solution to all reactions. Incubate for 20 min at 37° C. (This step usually increases the total number of colonies obtained by 10-20 fold.)
7. Transform 2  $\mu$ l into 100  $\mu$ l competent *E. coli*. Select on plates containing ampicillin at 100  $\mu$ g/ml.

#### **Example 7: Transformation of *E. coli***

To introduce cloning or Expression Vectors prepared using the recombinational cloning system of the invention, any standard *E. coli* transformation protocol should be satisfactory. The following steps are recommended for best results:

1. Let the mixture of competent cells and Recombinational Cloning System reaction product stand on ice at least 15 minutes prior to the heat-shock step. This gives time for the recombination proteins to dissociate from the DNA, and improves the transformation efficiency.

2. Expect the reaction to be about 1%-5% efficient, i.e., 2  $\mu$ l of the reaction should contain at least 100 pg of the Expression Clone plasmid (taking into account the amounts of each parental plasmid in the reaction, and the subsequent dilution). If the E. coli cells have a competence of  $10^7$  CFU/ $\mu$ g, 100 pg of the desired clone plasmid will give about 1000 colonies, or more, if the entire transformation is spread on one ampicillin plate.

3. Always do a control pUC DNA transformation. If the number of colonies is not what you expect, the pUC DNA transformation gives you an indication of where the problem was.

***Example 8: Preparation of attB-PCR Product***

For preparation of attB-PCR products in the PCR cloning methods described in Example 9 below, PCR primers containing attB1 and attB2 sequences are used. The attB1 and attB2 primer sequences are as follows:

**attB1:** 5'-GGGGACAAGTTTGTACAAAAAAGCAGGCT-(template-specific sequence)-3'

**attB2:** 5'-GGGGACCACTTTGTACAAGAAAGCTGGGT-(template-specific sequence)-3'

The attB1 sequence should be added to the amino primer, and the attB2 sequence to the carboxy primer. The 4 guanines at the 5' ends of each of these primers enhance the efficiency of the minimal 25 bp attB sequences as substrates for use in the cloning methods of the invention.

Standard PCR conditions may be used to prepare the PCR product. The following suggested protocol employs PLATINUM Taq DNA Polymerase High

-111-

Fidelity®, available commercially from Life Technologies, Inc. (Rockville, MD). This enzyme mix eliminates the need for hot starts, has improved fidelity over Taq, and permits synthesis of a wide range of amplicon sizes, from 200 bp to 10 kb, or more, even on genomic templates.

#### Materials needed:

- PLATINUM Taq DNA Polymerase High Fidelity® (Life Technologies, Inc.)
- attB1- and attB2- containing primer pair (see above) specific for your template
- DNA template (linearized plasmid or genomic DNA)
- 10X High Fidelity PCR Buffer
- 10 mM dNTP mix
- PEG/MgCl<sub>2</sub> Mix (30% PEG 8000, 30 mM MgCl<sub>2</sub>)

#### Procedure:

1.) Assemble the reaction as follows:

Component	Reaction with Plasmid Target	Reaction with <u>Genomic</u> Target
10X High Fidelity PCR Buffer	5 µl	5 µl
dNTP Mix 10 mM	1 µl	1 µl
MgSO <sub>4</sub> , 50mM	2 µl	2 µl
attB1 Primer, 10 µM	2 µl	1 µl
attB2 Primer, 10 µM	2 µl	1 µl
Template DNA	1-5 ng*	≥ 100 ng
PLATINUM Taq High Fidelity	2 µl	1 µl
Water	to 50 µl	to 50 µl

\* Use of higher amounts of plasmid template may permit fewer cycles (10-15) of PCR

2.) Add 2 drops mineral oil, as appropriate.

3.) Denature for 30 sec. at 94°C.

4.) Perform 25 cycles:

94°C for 15 sec-30 sec

55°C for 15 sec-30 sec

68°C for 1 min per kb of template.

5.) Following the PCR reaction, apply 1-2 µl of the reaction mixture to an agarose gel, together with size standards (*e.g.*, 1 Kb Plus Ladder, Life Technologies, Inc.) and quantitation standards (*e.g.*, Low Mass Ladder, Life Technologies, Inc.), to assess the yield and uniformity of the product.

Purification of the PCR product is recommended, to remove attB primer dimers which can clone efficiently into the Entry Vector. The following protocol is fast and will remove DNA <300 bp in size:

6.) Dilute the 50 µl PCR reaction to 200 µl with TE.

7.) Add 100 µl PEG/MgCl<sub>2</sub> Solution. Mix and centrifuge immediately at 13,000 RPM for 10 min at room temperature. Remove the supernatant (pellet is clear and hard to see).

8.) Dissolve the pellet in 50 µl TE and check recovery on a gel.

If the starting PCR template is a plasmid that contains the gene for Kan<sup>r</sup>, it is advisable to treat the completed PCR reaction with the restriction enzyme *DpnI*, to degrade the plasmid since unreacted residual starting plasmid is a potential source of false-positive colonies from the transformation of the GATEWAY™ Cloning System reaction. Adding ~5 units of *DpnI* to the completed PCR reaction and incubating for 15 min at 37°C will eliminate this potential problem. Heat inactivate the *DpnI* at 65°C for 15 min, prior to using the PCR product in the GATEWAY™ Cloning System reaction.



***Example 9: Cloning attB-PCR products into Entry Vectors via the BP ("Gateway") Reaction***

The addition of 5'-terminal attB sequences to PCR primers allows synthesis of a PCR product that is an efficient substrate for recombination with a Donor (attP) Plasmid in the presence of GATEWAY™ BP Clonase™ Enzyme Mix. This reaction produces an Entry Clone of the PCR product (See Figure 8).

The conditions of the Gateway Cloning reaction with an attB PCR substrate are similar to those of the BP Reaction (see Example 10 below), except that the attB-PCR product (see Example 8) substitutes for the Expression Clone, and the attB-PCR positive control (attB-tet<sup>r</sup>) substitutes for the Expression Clone Positive Control (GFP).

Materials needed:

- 5 X BP Reaction Buffer
- Desired attB-PCR product DNA, 50-100 ng in  $\leq 8 \mu\text{l}$  TE.
- Donor (attP) Plasmid (Figures 49-54), 75 ng/ $\mu\text{l}$ , supercoiled DNA
- attB-tet<sup>r</sup> PCR product positive control, 25 ng/ $\mu\text{l}$
- GATEWAY™ BP Clonase™ Enzyme Mix (stored at  $-80^{\circ}\text{C}$ )
- 10x Clonase Stop Solution
- pUC19 DNA, 10 pg/ $\mu\text{l}$ .
- Chemically competent E.coli cells (competence:  $\geq 1 \times 10^7$  CFU/ $\mu\text{g}$ ), 400  $\mu\text{l}$

Notes:

- Preparation of attB-PCR DNA: see Example 8.

• The Positive Control attB-tet<sup>r</sup> PCR product contains a functional copy of the tet<sup>r</sup> gene of pBR322, with its own promoter. By plating the transformation of the control BP Reaction on kanamycin (50  $\mu\text{g/ml}$ ) plates (if kan<sup>r</sup> Donor Plasmids are used; see Figures 49-52) or an alternative selection agent (e.g., gentamycin, if gen<sup>r</sup> Donor Plasmids are used; see Figure 54), and then picking about 50 of these colonies onto plates with tetracycline (20  $\mu\text{g/ml}$ ), the

percentage of Entry Clones containing functional tet<sup>r</sup> among the colonies from the positive control reaction can be determined (% Expression Clones = (number of tet<sup>r</sup> + kan<sup>r</sup> (or gen<sup>r</sup>) colonies/kan<sup>r</sup> (or gen<sup>r</sup>) colonies).

5

**Procedure:**

1. Assemble reactions as follows. Combine all components except GATEWAY™ BP Clonase™ Enzyme Mix, before removing GATEWAY™ BP Clonase™ Enzyme Mix from frozen storage.

10

	Neg.	Pos.	Test
Component	Tube 1	Tube 2	Tube 3
attB-PCR product, 50-100 ng			1 - 8 µl
Donor (attP) Plasmid 75 ng/µl	2 µl	2 µl	2 µl
attB-PCR tet <sup>r</sup> control DNA (75 ng/µl)		4 µl	
5 X BP Reaction Buffer	4 µl	4 µl	4 µl
TE	10 µl	6 µl	To 16 µl
GATEWAY™ BP Clonase™ Enzyme Mix (store at -80° C, add last)	4 µl	4 µl	4 µl
Total Volume	20 µl	20 µl	20 µl

15

20

25

30

2. Remove the GATEWAY™ BP Clonase™ Enzyme Mix from the -80° C freezer, place immediately on ice. The Clonase takes only a few minutes to thaw.
3. Add 4 µl of GATEWAY™ BP Clonase™ Enzyme Mix to the subcloning reaction, mix.
4. Return GATEWAY™ BP Clonase™ Enzyme Mix to - 80° C freezer.
5. Incubate tubes at 25° for at least 60 minutes.

6. Add 2  $\mu$ l Proteinase K (2  $\mu$ g/ $\mu$ l) to all reactions. Incubate for 20 min at 37°C.
7. Transform 2  $\mu$ l into 100  $\mu$ l competent *E. coli*, as per 3.2, above. Select on LB plates containing kanamycin, 50  $\mu$ g/ml.

#### Results:

In initial experiments, primers for amplifying tetR and ampR from pBR322 were constructed containing only the tetR- or ampR-specific targeting sequences, the targeting sequences plus attB1 (for forward primers) or attB2 (for reverse primers) sequences shown in Figure 9, or the attB1 or attB2 sequences with a 5' tail of four guanines. The construction of these primers is depicted in Figure 65. After PCR amplification of tetR and ampR from pBR322 using these primers and cloning the PCR products into host cells using the recombinational cloning system of the invention, the results shown in Figure 66 were obtained. These results demonstrated that primers containing attB sequences provided for a somewhat higher number of colonies on the tetracycline and ampicillin plates. However, inclusion of the 5' extensions of four or five guanines on the primers in addition to the attB sequences provided significantly better cloning results, as shown in Figures 66 and 67. These results indicate that the optimal primers for cloning of PCR products using recombinational cloning will contain the recombination site sequences with a 5' extension of four or five guanine bases.

To determine the optimal stoichiometry between attB-containing PCR products and attP-containing Donor plasmid, experiments were conducted where the amount of PCR product and Donor plasmid were varied during the BP Reaction. Reaction mixtures were then transformed into host cells and plated on tetracycline plates as above. Results are shown in Figure 68. These results indicate that, for optimal recombinational cloning results with a PCR product in the size range of the tet gene, the amounts of attP-containing Donor plasmids are between about 100-500 ng (most preferably about 200-300 ng), while the optimal concentrations of attB-containing PCR products is about 25-100 ng (most preferably about 100 ng), per 20  $\mu$ l reaction.

Experiments were then conducted to examine the effect of PCR product size on efficiency of cloning via the recombinational cloning approach of the invention.

PCR products containing attB1 and attB2 sites, at sizes 256 bp, 1 kb, 1.4 kb, 3.4 kb, 4.6 kb, 6.9 kb and 10.1 kb were prepared and cloned into Entry vectors as described above, and host cells were transformed with the Entry vectors containing the cloned PCR products. For each PCR product, cloning efficiency was calculated relative to cloning of pUC19 positive control plasmids as follows:

$$\text{Cloning Efficiency} = \frac{\text{CFU/ng attB PCR product}}{\text{CFU/ng pUC19 control}} \times \frac{\text{Size (kb) PCR product}}{\text{Size (kb) pUC19 control}}$$

The results of these experiments are depicted in Figures 69A-69C (for 256 bp PCR fragments), 70A-70C (for 1 kb PCR fragments), 71A-71C (for 1.4 kb PCR fragments), 72A-72C (for 3.4 kb PCR fragments), 73A-73C (for 4.6 kb PCR fragments), 74 (for 6.9 kb PCR fragments), and 75-76 (for 10.1 kb PCR fragments). The results shown in these figures are summarized in Figure 77, for different weights and moles of input PCR DNA.

Together, these results demonstrate that attB-containing PCR products ranging in size from about 0.25 kb to about 5 kb clone relatively efficiently in the recombinational cloning system of the invention. While PCR products larger than about 5 kb clone less efficiently (apparently due to slow resolution of cointegrates), longer incubation times during the recombination reaction appears to improve the efficiency of cloning of these larger PCR fragments. Alternatively, it may also be possible to improve efficiency of cloning of large (> about 5 kb) PCR fragments by using lower levels of input attP Donor plasmid and perhaps attB-containing PCR product, and/or by adjusting reaction conditions (e.g., buffer conditions) to favor more rapid resolution of the cointegrates.

#### ***Example 10: The BP Reaction***

One purpose of the Gateward ("Entry") reaction is to convert an Expression Clone into an Entry Clone. This is useful when you have isolated an individual Expression Clone from an Expression Clone cDNA library, and you wish to transfer the nucleic acid molecule of interest into another Expression Vector, or

to move a population of molecules from an attB or attL library. Alternatively, you may have mutated an Expression Clone and now wish to transfer the mutated nucleic acid molecule of interest into one or more new Expression Vectors. In both cases, it is necessary first to convert the nucleic acid molecule of interest to an Entry Clone.

Materials needed:

- 5 X BP Reaction Buffer
- Expression Clone DNA, 100-300 ng in  $\leq 8 \mu\text{l}$  TE.
- Donor (attP) Vector, 75 ng/ $\mu\text{l}$ , supercoiled DNA
- Positive control attB-tet-PCR DNA, 25 ng/ $\mu\text{l}$
- GATEWAY™ BP Clonase™ Enzyme Mix (stored at  $-80^\circ\text{C}$ )
- Clonase Stop Solution (Proteinase K, 2  $\mu\text{g}/\mu\text{l}$ ).

Notes:

Preparation of the Expression Clone DNA: Miniprep DNA treated with RNase works well.

1. As with the LR Reaction (see Example 14), the BP Reaction is strongly influenced by the topology of the reacting DNAs. In general, the reaction is most efficient when one of the DNAs is linear and the other is supercoiled, compared to reactions where the DNAs are both linear or both supercoiled. Further, linearizing the attB Expression Clone (anywhere within the vector) will usually give more colonies than linearizing the Donor (attP) Plasmid. If finding a suitable cleavage site within your Expression Clone vector proves difficult, you may linearize the Donor (attP) Plasmid between the attP1 and attP2 sites (for example, at the *NcoI* site), avoiding the *ccdB* gene. Maps of Donor (attP) Plasmids are given in Figures 49-54.

Procedure:

1. Assemble reactions as follows. Combine all components at room temperature, except GATEWAY™ BP Clonase™ Enzyme Mix, before removing GATEWAY™ BP Clonase™ Enzyme Mix from freezer.

	Neg.	Pos.	Test
Component	Tube 1	Tube 2	Tube 3
Positive Control, attB-tet-PCR DNA, 25 ng/ $\mu$ l	4 $\mu$ l	4 $\mu$ l	
Desired attB Expression Clone DNA (100ng) linearized			1 - 8 $\mu$ l
Donor (attP) Plasmid, 75 ng/ $\mu$ l	2 $\mu$ l	2 $\mu$ l	2 $\mu$ l
5 X BP Reaction Buffer	4 $\mu$ l	4 $\mu$ l	4 $\mu$ l
TE	10 $\mu$ l	6 $\mu$ l	To 16 $\mu$ l
GATEWAY™ BP Clonase™ Enzyme Mix (store at - 80° C, add last)	---	4 $\mu$ l	4 $\mu$ l
Total Volume	20 $\mu$ l	20 $\mu$ l	20 $\mu$ l

2. Remove the GATEWAY™ BP Clonase™ Enzyme Mix from the -80°C freezer, place immediately on ice. The mixture takes only a few minutes to thaw.
3. Add 4  $\mu$ l of GATEWAY™ BP Clonase™ Enzyme Mix to the subcloning reaction, mix.
4. Return GATEWAY™ BP Clonase™ Enzyme Mix to - 80° C freezer.
5. Incubate tubes at 25° for at least 60 minutes. If both the attB and attP DNAs are supercoiled, incubation for 2-24 hours at 25°C is recommended.
6. Add 2  $\mu$ l Clonase Stop Solution. Incubate for 10 min at 37°C.
7. Transform 2  $\mu$ l into 100  $\mu$ l competent E. coli, as above. Select on LB plates containing 50  $\mu$ g/ml kanamycin.

***Example 11: Cloning PCR Products into Entry Vectors using Standard Cloning Methods***

**Preparation of Entry Vectors for Cloning of PCR Products**

All of the Entry Vectors of the invention contain the death gene ccdB as a stuffer between the "left" and "right" restriction sites. The advantage of this arrangement is that there is virtually no background from vector that has not been cut with both restriction enzymes, because the presence of the ccdB gene will kill

all standard E. coli strains. Thus it is necessary to cut each Entry Vector twice, to remove the ccdB fragment.

We strongly recommend that, after digestion of the Entry Vector with the second restriction enzyme, you treat the reaction with phosphatase (calf intestine alkaline phosphatase, CIAP or thermosensitive alkaline phosphatase, TSAP). The phosphatase can be added directly to the reaction mixture, incubated for an additional time, and inactivated. This step dephosphorylates both the vector and ccdB fragments, so that during subsequent ligation there is less competition between the ccdB fragment and the DNA of interest for the termini of the Entry Vector.

#### Blunt Cloning of PCR products

Generally PCR products do not have 5' phosphates (because the primers are usually 5' OH), and they are not necessarily blunt. (On this latter point, see Brownstein, et al., *BioTechniques* 20: 1006, 1996 for a discussion of how the sequence of the primers affects the addition of single 3' bases.) The following protocol repairs these two defects.

In a 0.5 ml tube, ethanol precipitate about 40 ng of PCR product (as judged from an agarose gel).

1. Dissolve the precipitated DNA in 10  $\mu$ l comprising 1  $\mu$ l 10 mM rATP, 1  $\mu$ l mixed 2 mM dNTPs (i.e., 2 mM each dATP, dCTP, dTTP, and dGTP), 2  $\mu$ l 5x T4 polynucleotide kinase buffer (350 mM Tris HCl (pH7.6), 50 mM  $MgCl_2$ , 500mM KCl, 5 mM 2-mercaptoethanol) 10 units T4 polynucleotide kinase, 1  $\mu$ l T4 DNA polymerase, and water to 10  $\mu$ l.
2. Incubate the tube at 37° for 10 minutes, then at 65° for 15 minutes, cool, centrifuge briefly to bring any condensate to the tip of the tube.
3. Add 5  $\mu$ l of the PEG/ $MgCl_2$  solution, mix and centrifuge at room temperature for 10 minutes. Discard supernatant.
4. Dissolve the invisible precipitate in 10  $\mu$ l containing 2  $\mu$ l 5x T4 DNA ligase buffer (Life Technologies, Inc.), 0.5 units T4 DNA ligase, and about 50 ng of blunt, phosphatase-treated Entry Vector.

5. Incubate at 25° for 1 hour, then 65° for 10 minutes. Add 90 µl TE, transform 10 µl into 50 - 100 µl competent *E. coli* cells.
6. Plate on kanamycin.

5       **Note:** In the above protocol, steps b-c simultaneously polish the ends of the PCR product (through the exonuclease and polymerase activities of T4 DNA polymerase) and phosphorylate the 5' ends (using T4 polynucleotide kinase). It is necessary to inactivate the kinase, so that the blunt, dephosphorylated vector in step e cannot self ligate. Step d (the PEG precipitation) removes all small  
10       molecules (primers, nucleotides), and has also been found to improve the yield of cloned PCR product by 50 fold.

#### Cloning PCR Products after Digestion with Restriction Enzymes

Efficient cloning of PCR products that have been digested with restriction  
15       enzymes includes three steps: inactivation of *Taq* DNA polymerase, efficient restriction enzyme cutting, and removal of small DNA fragments.

*Inactivation of Taq DNA Polymerase:* Carryover of *Taq* DNA polymerase and dNTPs into a RE digestion significantly reduces the success in cloning a PCR product (D. Fox et al., *FOCUS* 20(1):15, 1998), because *Taq* DNA polymerase  
20       can fill in sticky ends and add bases to blunt ends. Either TAQQUENCH™ (obtainable from Life Technologies, Inc.; Rockville, Maryland) or extraction with phenol can be used to inactivate the *Taq*.

*Efficient Restriction Enzyme Cutting:* Extra bases on the 5' end of each PCR primer help the RE cut near ends of PCR products. With the availability of  
25       cheap primers, adding 6 to 9 bases on the 5' sides of the restriction sites is a good investment to ensure that most of the ends are digested. Incubation of the DNA with a 5-fold excess of restriction enzyme for an hour or more helps ensure success.

*Removal of Small Molecules before Ligation:* Primers, nucleotides,  
30       primer dimers, and small fragments produced by the restriction enzyme digestion,



can all inhibit or compete with the desired ligation of the PCR product to the cloning vector. This protocol uses PEG precipitation to remove small molecules.

Protocol for cutting the ends of PCR products with restriction enzyme(s):

5

1. Inactivation of Taq DNA polymerase in the PCR product:

Option A: Extraction with Phenol

10

A1. Dilute the PCR reaction to 200  $\mu$ l with TE. Add an equal volume of phenol:chloroform:isoamyl alcohol, vortex vigorously for 20 seconds, and centrifuge for 1 minute at room temperature. Discard the lower phase.

15

A2. Extract the phenol from the DNA and concentrate as follows. Add an equal volume of 2-butanol (colored red with "Oil Red O" from Aldrich, if desired), vortex briefly, centrifuge briefly at room temperature. Discard the upper butanol phase. Repeat the extraction with 2-butanol. This time the volume of the lower aqueous phase should decrease significantly. Discard the upper 2-butanol phase.

20

A3. Ethanol precipitate the DNA from the aqueous phase of the above extractions. Dissolve in a 200  $\mu$ l of a suitable restriction enzyme (RE) buffer.

Option B: Inactivation with TaqQuench

25

B1. Ethanol precipitate an appropriate amount of PCR product (100 ng to 1  $\mu$ g), dissolve in 200  $\mu$ l of a suitable RE buffer.

B2. Add 2  $\mu$ l TaqQuench.

30

2. Add 10 to 50 units of restriction enzyme and incubate for at least 1 hour. Ethanol precipitate if necessary to change buffers for digestion at the other end of the PCR product.

3. Add ½ volume of the PEG/MgCl<sub>2</sub> mix to the RE digestion. Mix well and immediately centrifuge at room temperature for 10 minutes. Discard the supernatant (pellet is usually invisible), centrifuge again for a few seconds, discard any remaining supernatant.

4. Dissolve the DNA in a suitable volume of TE (depending on the amount of PCR product in the original amplification reaction) and apply an aliquot to an agarose gel to confirm recovery. Apply to the same gel 20-100 ng of the appropriate Entry Vector that will be used for the cloning.

***Example 12: Determining The Expected Size of the GATEWAY™ Cloning Reaction Products***

If you have access to a software program that will electronically cut and splice sequences, you can create electronic clones to aid you in predicting the sizes and restriction patterns of GATEWAY™ Cloning System recombination products.

The cleavage and ligation steps performed by the enzyme Int in the GATEWAY™ Cloning System recombination reactions mimic a restriction enzyme cleavage that creates a 7-bp 5'-end overhang followed by a ligation step that reseals the ends of the daughter molecules. The recombination proteins present in the Clonase cocktails (see Example 19 below) recognize the 15 bp core sequence present within all four types of att sites (in addition to other flanking sequences characteristic of each of the different types of att sites).

By treating these sites in your software program as if they were restriction sites, you can cut and splice your Entry Clones with various Destination Vectors and obtain accurate maps and sequences of the expected results from your GATEWAY™ Cloning System reactions.

***Example 13: Protein Expression***

**Brief Review of Protein Expression**

*Transcription:* The most commonly used promoters in *E. coli* Expression Vectors are variants of the lac promoter, and these can be turned on by adding

IPTG to the growth medium. It is usually good to keep promoters off until expression is desired, so that the host cells are not made sick by the overabundance of some heterologous protein. This is reasonably easy in the case of the lac promoters used in *E. coli*. One needs to supply the *lac I* gene (or its more productive relative, the *lac I<sup>q</sup>* gene) to make *lac* repressor protein, which binds near the promoter and keeps transcription levels low. Some Destination Vectors for *E. coli* expression carry their own *lacI<sup>q</sup>* gene for this purpose. (However, lac promoters are always a little "on," even in the absence of IPTG.)

Controlling transcription in eukaryotic cells is not nearly so straightforward or efficient. The tetracycline system of Bujard and colleagues is the most successful approach, and one of the Destination Vectors (pDEST11; Figure 31) has been constructed to supply this function.

*Translation:* Ribosomes convert the information present in mRNA into protein. Ribosomes scan RNA molecules looking for methionine (AUG) codons, which begin nearly all nascent proteins. Ribosomes must, however, be able to distinguish between AUG codons that code for methionine in the middle of proteins from those at the start. Most often ribosomes choose AUGs that are 1) first in the RNA (toward the 5' end), and 2) have the proper sequence context. In *E. coli* the favored context (first recognized by Shine and Dalgarno, *Eur. J. Biochem.* 57: 221 (1975)) is a run of purines (As and Gs) from five to 12 bases upstream of the initiating AUG, especially AGGAGG or some variant.

In eukaryotes, a survey of translated mRNAs by Kozak (*J. Biol. Chem.* 266: 19867 (1991)) has revealed a preferred sequence context, gcc Acc ATGG, around the initiating methionine, with the A at -3 being most important, and a purine at +4 (where the A of the ATG is +1), preferably a G, being next most influential. Having an A at -3 is enough to make most ribosomes choose the first AUG of an mRNA, in plants, insects, yeast, and mammals. (For a review of initiation of protein synthesis in eukaryotic cells, see: Pain, V.M. *Eur.J. Biochem.* 236:747-771, 1996.)

*Consequences of Translation Signals for GATEWAY™ Cloning System:* First, translation signals (Shine-Dalgarno in *E. coli*, Kozak in eukaryotes) have to be close to the initiating ATG. The attB site is 25 base pairs long. Thus if

translation signals are desired near the natural ATG of the nucleic acid molecule of interest, they must be present in the Entry Clone of that nucleic acid molecule of interest. Also, when a nucleic acid molecule of interest is moved from an Entry Clone to a Destination vector, any translation signals will move along. The result is that the presence or absence of Shine-Dalgarno and/or Kozak sequences in the Entry Clone must be considered, with the eventual Destination Vectors to be used in mind.

Second, although ribosomes choose the 5' ATG most often, internal ATGs are also used to begin protein synthesis. The better the translation context around this internal ATG, the more internal translation initiation will be seen. This is important in the GATEWAY™ Cloning System, because you can make an Entry Clone of your nucleic acid molecule of interest, and arrange to have Shine-Dalgarno and/or Kozak sequences near the ATG. When this cassette is recombined into a Destination Vector that transcribes your nucleic acid molecule of interest, you get native protein. If you want, you can make a fusion protein in a different Destination Vector, since the Shine-Dalgarno and/or Kozak sequences do not contain any stop signals in the same reading frame. However, the presence of these internal translation signals may result in a significant amount of native protein being made, contaminating, and lowering the yield of, your fusion protein. This is especially likely with short fusion tags, like His6.

A good compromise can be recommended. If an Entry Vector like pENTR7 (Figure 16) or pENTR8 (Figure 17) is chosen, the Kozak bases are present for native eukaryotic expression. The context for *E. coli* translation is poor, so the yield of an amino-terminal fusion should be good, and the fusion protein can be digested with the TEV protease to make near-native protein following purification.

*Recommended Conditions for Synthesis of Proteins in E. coli:* When making proteins in *E. coli* it is advisable, at least initially, to incubate your cultures at 30°C, instead of at 37°C. Our experience indicates that proteins are less likely to form aggregates at 30°C. In addition, the yields of proteins from cells grown at 30°C frequently are improved.

The yields of proteins that are difficult to express may also be improved by inducing the cultures in mid-log phase of growth, using cultures begun in the morning from overnight growths, as opposed to harvesting directly from an overnight culture. In the latter case, the cells are preferably in late log or stationary growth, which can favor the formation of insoluble aggregates.

***Example 14: Constructing Destination Vectors from Existing Vectors***

Destination Vectors function because they have two recombination sites, attR1 and attR2, flanking a chloramphenicol resistance (CmR) gene and a death gene, ccdB. The GATEWAY™ Cloning System recombination reactions exchange the entire Cassette (except for a few bases comprising part of the attB sites) for the DNA segment of interest from the Entry Vector. Because attR1, CmR, ccdB gene, and attR2 are contiguous, they can be moved on a single DNA segment. If this Cassette is cloned into a plasmid, the plasmid becomes a Destination Vector. Figure 63 shows a schematic of the GATEWAY™ Cloning System Cassette; attR cassettes in all three reading frames contained in vectors pEZC15101, pEZC15102 and pEZC15103 are shown in Figures 64A, 64B, and 64C, respectively.

The protocol for constructing a Destination Vector is presented below. Keep in mind the following points:

- Destination Vectors must be constructed and propagated in one of the DB strains of *E. coli* (e.g., DB3.1, and particularly *E. coli* LIBRARY EFFICIENCY® DB3.1™ Competent Cells) available from Life Technologies, Inc. (and described in detail in U.S. Provisional Application No. 60/122,392, filed on March 2, 1999, which is incorporated herein by reference), because the ccdB death gene will kill any *E. coli* strain that has not been mutated such that it will survive the presence of the ccdB gene.
- If your Destination Vector will be used to make a fusion protein, a GATEWAY™ Cloning System cassette with the correct reading frame must be used. The nucleotide sequences of the ends of the cassettes are shown in Figure 78. The reading frame of the fusion protein domain must

be in frame with the core region of the attR1 site (for an amino terminal fusion) so that the six As are translated into two lysine codons. For a C-terminal fusion protein, translation through the core region of the attR2 site should be in frame with -TAC-AAA-, to yield -Tyr-Lys-.

- Note that each reading frame Cassette has a different unique restriction site between the chloramphenicol resistance and ccdB genes (*Mlu*I for reading frame A, *Bgl*II for reading frame B, and *Xba*I for reading frame C; see Figure 63).
- Most standard vectors can be converted to Destination Vectors, by inserting the Entry Cassette into the MCS of that vector.

#### Protocol for Making a Destination Vector

1. If the vector will make an amino fusion protein, it is necessary to keep the “aaa” triplets in attR1 in phase with the triplets of the fusion protein. Determine which Entry cassette to use as follows:

a.) Write out the nucleotide sequence of the existing vector near the restriction site into which the Entry cassette will be cloned. These must be written in triplets corresponding to the amino acid sequence of the fusion domain.

b.) Draw a vertical line through the sequence that corresponds to the restriction site end, after it has been cut and made blunt, i.e., after filling in a protruding 5' end or polishing a protruding 3' end.

c.) Choose the appropriate reading frame cassette:

- If the coding sequence of the blunt end ends after a complete codon triplet, use the reading frame A cassette. See Figures 78, 79 and 80.

- If the coding sequence of the blunt end ends in a single base, use the reading frame B cassette. See Figures 78, 79 and 81.

- If the coding sequence of the blunt end ends in two bases, use the reading frame C cassette. See Figures 78, 79, 82A-B, and 83A-C.

2. Cut one to five micrograms of the existing plasmid at the position where you wish your nucleic acid molecule of interest (flanked by att sites) to be after the recombination reactions. **Note:** it is better to remove as many of the MCS restriction sites as possible at this step. This makes it more likely that restriction enzyme sites within the GATEWAY™ Cloning System Cassette will be unique in the new plasmid, which is important for linearizing the Destination Vector (Example 14, below).

3. Remove the 5' phosphates with alkaline phosphatase. While this is not mandatory, it increases the probability of success.

4. Make the end(s) blunt with fill-in or polishing reactions. For example, to 1 µg of restriction enzyme-cut, ethanol-precipitated vector DNA, add:

- 20 µl 5x T4 DNA Polymerase Buffer (165 mM Tris-acetate (pH 7.9), 330 mM Na acetate, 50 mM Mg acetate, 500 µg/ml BSA, 2.5 mM DTT)
- 5 µl 10mM dNTP mix
- 1 Unit of T4 DNA Polymerase
- Water to a final volume of 100 µl
- Incubate for 15 min at 37°C.

5. Remove dNTPs and small DNA fragments: Ethanol precipitate (add three volumes of room temperature ethanol containing 0.1 M sodium acetate, mix well, immediately centrifuge at room temperature 5 - 10 minutes), dissolve wet precipitate in 200 µl TE, add 100 µl 30% PEG 8000, 30 mM MgCl<sub>2</sub>, mix well,

-128-

immediately centrifuge for 10 minutes at room temperature, discard supernatant, centrifuge again a few seconds, discard any residual liquid.

6. Dissolve the DNA to a final concentration of 10 - 50 ng per microliter. Apply  
20 - 100 ng to a gel next to supercoiled plasmid and linear size standards to  
confirm cutting and recovery. The cutting does not have to be 100% complete,  
since you will be selecting for the chloramphenicol marker on the Entry cassette.

7. In a 10  $\mu$ l ligation reaction combine 10 - 50 ng vector, 10 - 20 ng of Entry  
Cassette (Figure 79), and 0.5 units T4 DNA ligase in ligase buffer. After one hour  
(or overnight, whichever is most convenient), transform 1  $\mu$ l into one of the DB  
strains of competent *E. coli* cells with a *gyrA462* mutation (See U.S. Provisional  
Application No. 60/122,392, filed on March 2, 1999, which is incorporated herein  
by reference), preferably DB3.1, and most preferably *E. coli* LIBRARY  
EFFICIENCY@DB3.1™ Competent Cells. The *ccdB* gene on the Entry Cassette  
will kill other strains of *E. coli* that have not been mutated so as to survive the  
presence of the *ccdB* gene.

8. After expression in SOC medium, plate 10  $\mu$ l and 100  $\mu$ l on chloramphenicol-  
containing (30  $\mu$ g / ml) plates, incubate at 37° C.

9. Pick colonies, make miniprep DNA. Treat the miniprep with RNase A and  
store in TE. Cut with the appropriate restriction enzyme to determine the  
orientation of the Cassette. Choose clones with the attR1 site next to the amino  
end of the protein expression function of the plasmid.

#### Notes on Using Destination Vectors

- We have found that about ten-fold more colonies result from a GATEWAY™  
Cloning System reaction if the Destination Vector is linear or relaxed. If the  
competent cells you use are highly competent ( $>10^8$  per microgram),  
linearizing the Destination Vector is less essential.



- The site or sites used for the linearization must be within the Entry Cassette. Sites that cut once or twice within each cassette are shown in Figures 80-82.
- Minipreps of Destination Vectors will work fine, so long as they have been treated with RNase. Since most DB strains are *endA*- (See U.S. Provisional Application No. 60/122,392, filed on March 2, 1999, which is incorporated herein by reference), minipreps can be digested with restriction enzymes without a prior phenol extraction.
- Reading the OD<sub>260</sub> of miniprep DNA is inaccurate unless the RNA and ribonucleotides have been removed, for example, by a PEG precipitation.

***Example 15: Some Options in Choosing Appropriate Entry Vectors and Destination Vectors: An Example***

In some applications, it may be desirable to express a nucleic acid molecule of interest in two forms: as an amino-terminal fusion in *E. coli*, and as a native protein in eukaryotic cells. This may be accomplished in any of several ways:

**Option 1:** Your choices depend on your nucleic acid molecule of interest and the fragment that contains it, as well as the available Entry Vectors. For eukaryotic translation, you need consensus bases according to Kozak (*J. Biol. Chem.* 266:19867, 1991) near the initiating methionine (ATG) codon. All of the Entry Vectors offer this motif upstream of the *Xmn*I site (blunt cutter). One option is to amplify your nucleic acid molecule of interest, with its ATG, by PCR, making the amino end blunt and the carboxy end containing the natural stop codon followed by one of the "right side" restriction sites (*Eco*RI, *Not*I, *Xho*I, *Eco*RV, or *Xba*I of the pENTR vectors).

If you know your nucleic acid molecule of interest does not have, for example, an *Xho*I site, you can make a PCR product that has this structure:

**Xho I**

```
5' ATG nnn nnn --- nnn TAA ctc gag nnn nnn 3'
3' tac nnn nnn --- nnn att gag ctc nnn nnn 5'
```

After cutting with *Xho*I, the fragment is ready to clone:

```
5' ATG nnn nnn --- nnn TAA c      3'
3' tac nnn nnn --- nnn att gag ct 5'
```

5 (If you follow this example, don't forget to put a phosphate on the amino oligo.)

**Option 2:** This PCR product could be cloned into two Entry Vectors to give the desired products, between the *Xmn*I and *Xho*I sites: pENTR1A (Figures 10A, 10B ) or pENTR7 (Figures 16A, 16B). If you clone into pENTR1A, amino fusions will have the minimal number of amino acids between the fusion domain and your nucleic acid molecule of interest, but the fusion cannot be removed with TEV protease. The converse is true of clones in pENTR7, i.e., an amino fusion can be cleaved with TEV protease, at the cost of more amino acids between the fusion and your nucleic acid molecule of interest.

15 In this example, let us choose to clone our hypothetical nucleic acid molecule of interest into pENTR7, between the *Xmn*I and *Xho*I sites. Once this is accomplished, several optional protocols using the Entry Clone pENTR7 may be followed:

20 **Option 3:** Since the nucleic acid molecule of interest has been amplified with PCR, it may be desirable to sequence it. To do this, transfer the nucleic acid molecule of interest from the Entry Vector into a vector that has priming sites for the standard sequencing primers. Such a vector is pDEST6 (Figures 26A, 26B). This Destination Vector places the nucleic acid molecule of interest in the opposite orientation to the lac promoter (which is leaky -- see Example 3 above). If the gene product is toxic to *E. coli*, this Destination Vector will minimize its toxicity.

25 **Option 4:** While the sequencing is going on, you might wish to check the expression of the nucleic acid molecule of interest in, for example, CHO cells, by recombining the nucleic acid molecule of interest into a CMV promoter vector (pDEST7, Figure 27; or pDEST12, Figure 32), or into a baculovirus vector (pDEST8, Figure 28; or pDEST10, Figure 30) for expression in insect cells. Both

of these vectors will transcribe the coding sequence of your nucleic acid molecule of interest, and translate it from the ATG of the PCR product using the Kozak bases upstream of the *XmnI* site.

**Option 5:** If you wish to purify protein, for example to make antibodies, you can clone the nucleic acid molecule of interest into a His6 fusion vector, pDEST2 (Figure 22). Since the nucleic acid molecule of interest is cloned downstream of the TEV protease cleavage domain of pENTR7 (Figure 16), the amino acid sequence of the protein produced will be:

10 [----- attB1 -----] TEV protease  
NH2- MSYYHHHHHHGITSLYKKAGFENLYFQIGTM----COOH

The attB site and the restriction sites used to make the Destination and Entry Vectors are translated into the underlined 11 amino acids (GITSLYKKAGF). Cleavage with TEV protease (arrow) leaves two amino acids, GT, on the amino end of the gene product.

See Figure 55 for an example of a nucleic acid molecule of interest, the chloramphenicol acetyl transferase (CAT) gene, cloned into pENTR7 (Figure 16) as a blunt (amino)-*Xho*I (carboxy) fragment, then cloned by recombination into the His6 fusion vector pDEST2 (Figure 22).

**Option 6:** If the His6 fusion protein is insoluble, you may go on and try a GST fusion. The appropriate Destination vector is pDEST3 (Figure 23).

25      **Option 7:** If you need to make RNA probes and prefer SP6 RNA polymerase, you can make the top strand RNA with your nucleic acid molecule of interest cloned into pSPORT+ (pDEST5 (Figures 25A, 25B)), and the bottom strand RNA with the nucleic acid molecule of interest cloned into pSPORT(-) (pDEST6 (Figures 26A, 26B)). Opposing promoters for T7 RNA polymerase and SP6 RNA  
30      polymerase are also present in these clones.

**Option 8:** It is often worthwhile to clone your nucleic acid molecule of interest into a variety of Destination Vectors in the same experiment. For example, if the number of colonies varies widely when the various recombination reactions are transformed into *E. coli*, this may be an indication that the nucleic acid molecule of interest is toxic in some contexts. (This problem is more clearly evident when a positive control gene is used for each Destination Vector.) Specifically, if many more colonies are obtained when the nucleic acid molecule of interest is recombined into pDEST6 than in pDEST5, there is a good chance that leakiness of the lac promoter is causing some expression of the nucleic acid molecule of interest in pSPORT “+” (which is not harmful in pDEST6 because the nucleic acid molecule of interest is in the opposite orientation).

***Example 16: Demonstration of a One-tube Transfer of a PCR Product (or Expression Clone) to Expression Clone via a Recombinational Cloning Reaction***

In the BxP recombination (Entry or Gateway) reaction described herein, a DNA segment flanked by attB1 and attB2 sites in a plasmid conferring ampicillin resistance was transferred by recombination into an attP plasmid conferring kanamycin resistance, which resulted in a product molecule wherein the DNA segment was flanked by attL sites (attL1 and attL2). This product plasmid comprises an “attL Entry Clone” molecule, because it can react with a “attR Destination Vector” molecule via the LxR (Destination) reaction, resulting in the transfer of the DNA segment to a new (ampicillin resistant) vector. In the previously described examples, it was necessary to transform the BxP reaction products into *E. coli*, select kanamycin resistant colonies, grow those colonies in liquid culture, and prepare miniprep DNA, before reacting this DNA with a Destination Vector in an LxR reaction.

The goal of the following experiment was to eliminate the transformation and miniprep DNA steps, by adding the BxP Reaction products directly to an LxR Reaction. This is especially appropriate when the DNA segment flanked by attB sites is a PCR product instead of a plasmid, because the PCR product cannot give

ampicillin-resistant colonies upon transformation, whereas attB plasmids (in general) carry an ampicillin resistance gene. Thus use of a PCR product flanked by attB sites in a BxP Reaction allows one to select for the ampicillin resistance encoded by the desired attB product of a subsequent LxR Reaction.

Two reactions were prepared: Reaction A, negative control, no attB PCR product, (8 µl) contained 50 ng pEZC7102 (attP Donor plasmid, confers kanamycin resistance) and 2 µl BxP Clonase (22 ng / µl Int protein and 8 ng/µl IHF protein) in BxP buffer (25 mM Tris HCl, pH 7.8, 70 mM KCl, 5 mM spermidine, 0.5 mM EDTA, 250 µg / ml BSA). Reaction B (24 µl) contained 150 ng pEZC7102, 6 µl BxP Clonase, and 120 ng of the attB -tet-PCR product in the same buffer as reaction A. The attB - tet - PCR product comprised the tetracycline resistance gene of plasmid pBR322, amplified with two primers containing either attB1 or attB2 sites, and having 4 Gs at their 5' ends, as described earlier.

The two reactions were incubated at 25°C for 30 minutes. Then aliquots of these reactions were added to new components that comprised LxR Reactions or appropriate controls for the LxR Reaction. Five new reactions were thus produced:

**Reaction 1:** 5 µl of reaction A was added to a 5 µl LxR Reaction containing 25 ng NcoI-cut pEZC8402 (the attR Destination Vector plasmid) in LxR buffer (37.5 mM Tris HCl, pH 7.7, 16.5 mM NaCl, 35 mM KCl, 5 mM spermidine, 375 µg / ml BSA), and 1 µl of GATEWAY™ LR Clonase™ Enzyme Mix (total volume of 10 µl).

**Reaction 2:** Same as reaction 1, except 5 µl of reaction B (positive) were added instead of reaction A (negative).

**Reaction 3:** Same as reaction 2, except that the amounts of Nco-cut pEZC8402 and GATEWAY™ LR Clonase™ Enzyme Mix were doubled, to 50 ng and 2 µl, respectively.

**Reaction 4:** Same as reaction 2, except that 25 ng of pEZ11104 (a positive control attL Entry Clone plasmid) were added in addition to the aliquot of reaction B.

**Reaction 5:** Positive control LxR Reaction, containing 25 ng *Nco*I-cut pEZX8402, 25 ng pEZ11104, 37.5 mM Tris HCl pH 7.7, 16.5 mM NaCl, 35 mM KCl, 5 mM spermidine, 375 µg / ml BSA and 1 µl GATEWAY™ LR Clonase™ Enzyme Mix in a total volume of 5 µl.

All five reactions were incubated at 25°C for 30 minutes. Then, 1 µl aliquots of each of the above five reactions, plus 1 µl from the remaining volume of Reaction B, the standard BxP Reaction, were used to transform 50 µl competent DH5α *E. coli*. DNA and cells were incubated on ice for 15 min., heat shocked at 42°C for 45 sec., and 450 µl SOC were added. Each tube was incubated with shaking at 37°C for 60 min. Aliquots of 100 µl and 400 µl of each transformation were plated on LB plates containing either 50 µg/ml kanamycin or 100 µg/ml ampicillin (see Table 2). A transformation with 10 pg of pUC19 DNA (plated on LB-amp<sub>100</sub>) served as a control on the transformation efficiency of the DH5α cells. Following incubation overnight at 37°C, the number of colonies on each plate was determined.

Results of these reactions are shown in Table 2.

**Table 2\***

Reaction No.:	1	2	3	4	5	6
	Number of Colonies					
Vol. plated:	Neg. Control BxP Reaction	1X pEZC8402 and LR Clonase™	2X pEZC8402 and LR Clonase™	LxR Reaction with Pos. Control DNA	LxR Reaction alone	BxP Reaction alone
100 µl	2	1	8	9	~1000	~1000
400 µl	5	10	35	62	>2000	>2000
Selection:	Kan	Amp	Amp	Amp	Amp	Kan

\*(Transformation with pUC 19 DNA yielded  $1.4 \times 10^9$  CFU/µg DNA.)

34 of the 43 colonies obtained from Reaction 3 were picked into 2 ml Terrific Broth with 100 µg/ml ampicillin and these cultures were grown overnight, with shaking, at 37°C. 27 of the 34 cultures gave at least moderate growth, and of these 24 were used to prepare miniprep DNA, using the standard protocol. These 24 DNAs were initially analyzed as supercoiled (SC) DNA on a 1% agarose gel to identify those with inserts and to estimate the sizes of the inserts. Fifteen of the 24 samples displayed SC DNA of the size predicted (5553 bp) if **tetx7102** had correctly recombined with **pEZC8402** to yield **tetx8402**. One of these samples contained two plasmids, one of ~5500 bp and a one of ~3500 bp. The majority of the remaining clones were approximately 4100 bp in size.

All 15 of the clones displaying SC DNA of predicted size (~5500 bp) were analyzed by two different double digests with restriction endonucleases to confirm the structure of the expected product: **tetx8402**. (See plasmid maps, Figures 57-59) In one set of digests, the DNAs were treated with Not I and Eco RI, which should cut the predicted product just outside both attB sites, releasing the tet<sup>r</sup> insert on a fragment of 1475 bp. In the second set of digests, the DNAs were digested with *NotI* and with *NruI*. *NruI* cleaves asymmetrically within the subcloned tet<sup>r</sup> insert, and together with *NotI* will release a fragment of 1019 bp.

Of the 15 clones analyzed by double restriction digestion, 14 revealed the predicted sizes of fragments for the expected product.

#### **Interpretation:**

The DNA components of Reaction B, pEZC7102 and attB-tet-PCR, are shown in Figure 56. The desired product of BxP Reaction B is tetx7102, depicted in Figure 57. The LxR Reaction recombines the product of the BxP Reaction, tetx7102 (Figure 57), with the Destination Vector, pEZC8402, shown in Figure 58. The LxR Reaction with tetx7102 plus pEZC8402 is predicted to yield the desired product tetx8402, shown in Figure 59.

Reaction 2, which combined the BxP Reaction and LxR Reaction, gave few colonies beyond those of the negative control Reaction. In contrast, Reaction 3, with twice the amount of pEZC8402 (Figure 58) and LxR Clonase, yielded a

larger number of colonies. These colonies were analyzed further, by restriction digestion, to confirm the presence of expected product. Reaction 4 included a known amount of attL Entry Clone plasmid in the combined BxP-plus-LxR reaction. But reaction 4 yielded only about 1% of the colonies obtained when the same DNA was used in a LxR reaction alone, Reaction 6. This result suggests that the LxR reaction may be inhibited by components of the BxP reaction.

Restriction endonuclease analysis of the products of Reaction 3 revealed that a sizeable proportion of the colonies (14 of the 34 analyzed) contained the desired tet<sup>r</sup> subclone, tetx8402 (Figure 59).

The above results establish the feasibility of performing first a BxP recombination reaction followed by a LxR recombination reaction -- in the same tube -- simply by adding the appropriate buffer mix, recombination proteins, and DNAs to a completed BxP reaction. This method should prove useful as a faster method to convert attB-containing PCR products into different Expression Clones, eliminating the need to isolate first the intermediate attL-PCR insert subclones, before recombining these with Destination Vectors. This may prove especially valuable for automated applications of these reactions.

This same one-tube approach allows for the rapid transfer of nucleic acid molecules contained in attB plasmid clones into new functional vectors as well. As in the above examples, attL subclones generated in a BxP Reaction can be recombined directly with various Destination Vectors in a LxR reaction. The only additional requirement for using attB plasmids, instead of attB-containing PCR products, is that the Destination Vector(s) employed must contain a different selection marker from the one present on the attB plasmid itself and the attP vector.

Two alternative protocols for a one-tube reaction have also proven useful and somewhat more optimal than the conditions described above.

#### Alternative 1:

Reaction buffer contained 50 mM Tris-HCl (pH 7.5), 50 mM NaCl, 0.25 mM EDTA, 2.5 mM spermidine, and 200 µg/ml BSA. After a 16 (or 3) hour incubation of the PCR product (100 ng) + attP Donor plasmid (100 ng) +



GATEWAY™ BP Clonase™ Enzyme Mix + Destination Vector (100 ng), 2 µl of GATEWAY™ LR Clonase™ Enzyme Mix (per 10 µl reaction mix) was added and the mixture was incubated an additional 6 (or 2) hours at 25°C. Stop solution was then added as above and the mixture was incubated at 37°C as above and transformed by electroporation with 1 µl directly into electrocompetent host cells. Results of this series of experiments demonstrated that longer incubation times (16 hours vs. 3 hours for the BP Reaction, 6 hours vs. 2 hours for the LR Reaction) resulted in about twice as many colonies being obtained as for the shorter incubation times. With two independent genes, 10/10 colonies having the correct cloning patterns were obtained.

Alternative 2:

A standard BP Reaction under the reaction conditions described above for Alternative 1 was performed for 2 hours at 25°C. Following the BP Reaction, the following components were added to the reaction mixture in a total volume of 7 µl:

20 mM Tris-HCl, pH 7.5  
100 mM NaCl  
5 µg/ml Xis-His6  
15% glycerol  
~1000 ng of Destination Vector

The reaction mixture was then incubated for 2 hours at 25°C, and 2.5 µl of stop solution (containing 2 µg/ml proteinase K) was added and the mixture was incubated at 37°C for an additional 10 minutes. Chemically competent host cells were then transformed with 2 µl of the reaction mixture, or electrocompetent host cells (*e.g.*, EMax DH10B cells; Life Technologies, Inc.) were electroporated with 2 µl of the reaction mixture per 25-40 µl of cells. Following transformation, mixtures were diluted with SOC, incubated at 37°C, and plated as described above on media selecting for the selection markers on the Destination Vector and the Entry clone (B x P reaction product). Analogous results to those described for Alternative 1 were obtained with these reaction conditions -- a higher level of colonies containing correctly recombined reaction products were observed.

***Example 17: Demonstration of a One-tube Transfer of a PCR Product (or Expression Clone) to Expression Clone via a Recombinational Cloning Reaction***

5                   Single-tube transfer of PCR product DNA or Expression Clones into Expression Clones by recombinational cloning has also been accomplished using a procedure modified from that described in Example 16. This procedure is as follows:

10                   •Perform a standard BP (Gateway) Reaction (see Examples 9 and 10) in 20 µl volume at 25°C for 1 hour.

15                   •After the incubation is over, take a 10 µl aliquot from the 20 µl total volume and add 1 µl of Proteinase K (2 mg/ml) and incubate at 37°C for 10 minutes. This first aliquot can be used for transformation and gel assay of BP reaction analysis. Plate BP reaction transformation on LB plates with **Kanamycin** (50 ug/ml).

20                   •Add the following reagents to the remaining 10 µl aliquot of the BP reaction:

1 µl of 0.75 M NaCl

2 µl of destination vector (150 ng/µl)

4 µl of LR Clonase™ (after thawing and brief mixing)

25                   •Mix all reagents well and incubate at 25°C for 3 hours. Stop the reaction at the end of incubation with 1.7 µl of Proteinase K (2 mg/ml) and incubate at 37°C for 10 minutes.

30                   •Transform 2 µl of the completed reaction into 100 µl of competent cells. Plate 100 µl and 400 µl on LB plates with **Ampicillin** (100 µg/ml).

**Notes:**

•If your competent cells are less than 10<sup>8</sup> CFU/µg, and you are concerned about getting enough colonies, you can improve the yield several fold by incubating the

-139-

BP reaction for 6-20 hours. Electroporation also can yield better colony output than chemical transformation.

•PCR products greater than about 5-6 kb show significantly lower cloning efficiency in the BP reaction. In this case, we recommend using longer incubation times for both BP and LR steps.

•If you want to move your insert gene into several destination vectors simultaneously, then scale up the initial BP reaction volume so that you have a 10 µl aliquot for adding each destination vector.

***Example 18: Optimization of GATEWAY™ Clonase™ Enzyme Compositions***

The enzyme compositions containing Int and IHF (for BP Reactions) were optimized using a standard functional recombinational cloning reaction (a BP reaction) between attB-containing plasmids and attP-containing plasmids, according to the following protocol:

**Materials and Methods:**

***Substrates:***

AttP - supercoiled pDONR201

AttB - linear ~ 1Kb [<sup>3</sup>H]PCR product amplified from pEZX7501

***Proteins:***

IntH6 -- His<sub>6</sub>-carboxy- tagged λ Integrase

IHF -- Integration Host Factor

***Clonase:***

50 ng/µl IntH6 and 20 ng/µl IHF, admixed in 25 mM Tris- HCl (pH 7.5), 22 mM NaCl, 5 mM EDTA, 1 mg/ml BSA, 5 mM Spermidine, and 50% glycerol.

*Reaction Mixture (total volume of 40  $\mu$ l):*

1000 ng AttP plasmid

600 ng AttB [ $^3$ H] PCR product

8  $\mu$ l Clonase (400 ng IntH6, 160 ng IHF) in 25 mM Tris-HCl (pH 7.5),  
22 mM NaCl, 5 mM EDTA, 1 mg/ml BSA, 5 mM Spermidine, 5 mM  
DTT.

Reaction mixture was incubated for 1 hour at 25°C, 4  $\mu$ l of 2  $\mu$ g/ $\mu$ l  
proteinase K was added and mixture was incubated for an additional 20 minutes  
at 37°C. Mixture was then extracted with an equal volume of Phenol/Chloroform/  
Isoamyl alcohol. The aqueous layer was then collected, and 0.1 volumes of 3 M  
sodium acetate and 2 volumes of cold 100% ethanol were added. Tubes were  
then spun in a microcentrifuge at maximum RPM for 10 minutes at room  
temperature. Ethanol was decanted, and pellets were rinsed with 70% ethanol and  
re-centrifuged as above. Ethanol was decanted, and pellets were allowed to air  
dry for 5-10 minutes and then dissolved in 20  $\mu$ l of 33 mM Tris-Acetate (pH 7.8),  
66 mM potassium acetate, 10 mM magnesium acetate, 1 mM DTT, and 1mM  
ATP. 2 units of exonuclease V (e.g., Plasmid Safe; EpiCentre, Inc., Madison, WI)  
was then added, and the mixture was incubated at 37°C for 30 minutes.

Samples were then TCA-washed by spotting 30  $\mu$ l of reaction mixture  
onto a Whatman GF/C filter, washing filters once with 10% TCA + 1% NaPPi for  
10 minutes, three times with 5% TCA for 5 minutes each, and twice with ethanol  
for 5 minutes each. Filters were then dried under a heat lamp, placed into a  
scintillation vial, and counted on a  $\beta$  liquid scintillation counter (LSC).

The principle behind this assay is that, after exonuclease V digestion, only  
double-stranded circular DNA survives in an acid-insoluble form. All DNA  
substrates and products that have free ends are digested to an acid-soluble form  
and are not retained on the filters. Therefore, only the  $^3$ H-labeled attB linear DNA  
which ends up in circular form after both inter- and intramolecular integration is  
complete is resistant to digestion and is recovered as acid-insoluble product.  
Optimal enzyme and buffer formulations in the Clonase compositions therefore are  
those that give the highest levels of circularized  $^3$ H-labeled attB-containing

sequences, as determined by highest cpm in the LSC. Although this assay was designed for optimization of GATEWAY™ BP Clonase™ Enzyme Mix compositions (Int + IHF), the same type of assay may be performed to optimize GATEWAY™ LR Clonase™ Enzyme Mix compositions (Int + IHF + Xis), except that the reaction mixtures would comprise 1000 ng of AttR (instead of AttP) and 600 ng of AttL (instead of AttB), and 40 ng of His<sub>6</sub>-carboxy- tagged Xis (XisH6) in addition to the IntH6 and IHF.

***Example 19: Testing Functionality of Entry and Destination Vectors***

As part of assessment of the functionality of particular vectors of the invention, it is important to functionally test the ability of the vectors to recombine. This assessment can be carried out by performing a recombinational cloning reaction (as schematized in Figures 2, 4, and 5A and 5B, and as described herein and in commonly owned U.S. Application Nos. 08/486,139, filed June 7, 1995, 08/663,002, filed June 7, 1996 (now U.S. Patent No. 5,888,732), 09/005,476, filed January 12, 1998, and 09/177,387, filed October 23, 1998, the disclosures of all of which are incorporated by reference herein in their entireties), by transforming E. coli and scoring colony forming units. However, an alternative assay may also be performed to allow faster, more simple assessment of the functionality of a given Entry or Destination Vector by agarose gel electrophoresis. The following is a description of such an in vitro assay.

**Materials and Methods:**

Plasmid templates pEZC1301 (Figure 84) and pEZC1313 (Figure 85), each containing a single wild type att site, were used for the generation of PCR products containing attL or attR sites, respectively. Plasmid templates were linearized with *A*lwNI, phenol extracted, ethanol precipitated and dissolved in TE to a concentration of 1 ng/μl.

PCR primers (capital letters represent base changes from wildtype):

attL1            gggg agcct gctttttGtacAaa gttggcatta taaaaagca ttgc  
attL2            gggg agcct gctttCttGtacAaa gttggcatta taaaaagca ttgc  
attL right        tgttgccggg aagctagagt aa

5

attR1            gggg Acaag ttTgtaCaaaaaagc tgaacgaga aacgtaaaat  
attR2            gggg Acaag ttTgtaCaaGaaagc tgaacgaga aacgtaaaat  
attR right        ca gacggcatga tgaacctgaa

10

PCR primers were dissolved in TE to a concentration of 500 pmol/μl. Primer mixes were prepared, consisting of attL1 + attLright primers, attL2 + attLright primers, attR1 + attRright primers, and attR2 + attRright primers, each mix containing 20 pmol/μl of each primer.

PCR reactions:

15

1 μl plasmid template (1 ng)  
1 μl primer pairs (20 pmoles of each)  
3 μl of H<sub>2</sub>O  
45 μl of Platinum PCR SuperMix® (Life Technologies, Inc.)

20

Cycling conditions (performed in MJ thermocycler):

95°C/2 minutes  
94°C/30 seconds  
25 cycles of 58°C/30 seconds and 72°C/1.5 minutes  
72°C/5 minutes  
5°C/hold

25

The resulting attL PCR product was 1.5 kb, and the resulting attR PCR product was 1.0 kb.

30

PCR reactions were PEG/MgCl<sub>2</sub> precipitated by adding 150 μl H<sub>2</sub>O and 100 μl of 3x PEG/ MgCl<sub>2</sub> solution followed by centrifugation. The PCR products were dissolved in 50 μl of TE. Quantification of the PCR product was performed by gel electrophoresis of 1 μl and was estimated to be 50-100 ng/μl.

Recombination reactions of PCR products containing attL or attR sites with GATEWAY™ plasmids was performed as follows:

8 µl of H<sub>2</sub>O

2 µl of attL or attR PCR product (100-200 ng)

5 2 µl of GATEWAY™ plasmid (100 ng)

4 µl of 5x Destination buffer

4 µl of GATEWAY™ LR Clonase™ Enzyme Mix

20 µl total volume (the reactions can be scaled down to a 5 µl total volume by adjusting the volumes of the components to about ¼ of those shown above, while keeping the stoichiometries the same).

10 Clonase reactions were incubated at 25°C for 2 hours. 2 µl of proteinase K (2 mg/ml) was added to stop the reaction. 10 µl was then run on a 1 % agarose gel. Positive control reactions were performed by reacting attL1 PCR product (1.0 kb) with attR1 PCR product (1.5 kb) and by similarly reacting attL2 PCR product with attR2 PCR product to observe the formation of a larger (2.5 kb) recombination product. Negative controls were similarly performed by reacting attL1 PCR product with attR2 PCR product and vice versa or reactions of attL PCR product with an attL plasmid, etc.

20 In alternative assays, to test attB Entry vectors, plasmids containing single attP sites were used. Plasmids containing single att sites could also be used as recombination substrates in general to test all Entry and Destination vectors (*i.e.*, those containing attL, attR, attB and attP sites). This would eliminate the need to do PCR reactions.

25 Results:

Destination and Entry plasmids when reacted with appropriate att-containing PCR products formed linear recombinant molecules that could be easily visualized on an agarose gel when compared to control reactions containing no attL or attR PCR product. Thus, the functionality of Destination and Entry vectors constructed according to the invention may be determined either by carrying out the Destination or Entry recombination reactions as depicted in

Figures 2, 4, and 5A and 5B, or more rapidly by carrying out the linearization assay described in this Example.

***Example 20: PCR Cloning Using Universal Adapter-Primers***

As described herein, the cloning of PCR products using the GATEWAY™ PCR Cloning System (Life Technologies, Inc.; Rockville, MD) requires the addition of attB sites (attB1 and attB2) to the ends of gene-specific primers used in the PCR reaction. The protocols described in the preceding Examples suggest that the user add 29 bp (25 bp containing the attB site plus four G residues) to the gene-specific primer. It would be advantageous to high volume users of the GATEWAY™ PCR Cloning System to generate attB-containing PCR product using universal attB adapter-primers in combination with shorter gene-specific primers containing a specified overlap to the adapters. The following experiments demonstrate the utility of this strategy using universal attB adapter-primers and gene-specific primers containing overlaps of various lengths from 6 bp to 18 bp. The results demonstrate that gene-specific primers with overlaps of 10 bp to 18 bp can be used successfully in PCR amplifications with universal attB adapter-primers to generate full-length PCR products. These PCR products can then be successfully cloned with high fidelity in a specified orientation using the GATEWAY™ PCR Cloning System.

**Methods and Results:**

To demonstrate that universal attB adapter-primers can be used with gene-specific primers containing partial attB sites in PCR reactions to generate full-length PCR product, a small 256 bp region of the human hemoglobin cDNA was chosen as a target so that intermediate sized products could be distinguished from full-length products by agarose gel electrophoresis.

The following oligonucleotides were used:

B1-Hgb: GGGG ACA AGT TTG TAC AAA AAA GCA GGC T-5'-Hgb\*  
B2-Hgb: GGGG ACC ACT TTG TAC AAG AAA GCT GGG T-3'-Hgb\*\*



-145-

18B1-Hgb: TG TAC AAA AAA GCA GGC T-5'-Hgb  
 18B2-Hgb: TG TAC AAG AAA GCT GGG T-3'-Hgb  
 15B1-Hgb: AC AAA AAA GCA GGC T-5'-Hgb  
 15B2-Hgb: AC AAG AAA GCT GGG T-3'-Hgb  
 5 12B1-Hgb: AA AAA GCA GGC T-5'-Hgb  
 12B2-Hgb: AG AAA GCT GGG T-3'-Hgb  
 11B1-Hgb: A AAA GCA GGC T-5'-Hgb  
 11B2-Hgb: G AAA GCT GGG T-3'-Hgb  
 10B1-Hgb: AAA GCA GGC T-5'-Hgb  
 10 10B2-Hgb: AAA GCT GGG T-3'-Hgb  
 9B1-Hgb: AA GCA GGC T-5'-Hgb  
 9B2-Hgb: AA GCT GGG T-3'-Hgb  
 8B1-Hgb: A GCA GGC T-5'-Hgb  
 8B2-Hgb: A GCT GGG T-3'-Hgb  
 15 7B1-Hgb: GCA GGC T-5'-Hgb  
 7B2-Hgb: GCT GGG T-3'-Hgb  
 6B1-Hgb: CA GGC T-5'-Hgb  
 6B2-Hgb: CT GGG T-3'-Hgb  
  
 20 attB1 adapter: GGGG ACA AGT TTG TAC AAA AAA GCA GGC T  
 attB2 adapter: GGGG ACC ACT TTG TAC AAG AAA GCT GGG T  
  
 \* -5'-Hgb = GTC ACT AGC CTG TGG AGC AAG A  
 \*\* -3'-Hgb = AGG ATG GCA GAG GGA GAC GAC A

25

The aim of these experiments was to develop a simple and efficient universal adapter PCR method to generate attB containing PCR products suitable for use in the GATEWAY™ PCR Cloning System. The reaction mixtures and thermocycling conditions should be simple and efficient so that the universal adapter PCR method could be routinely applicable to any PCR product cloning application.

30

PCR reaction conditions were initially found that could successfully amplify predominately full-length PCR product using gene-specific primers containing 18bp and 15 bp overlap with universal attB primers. These conditions are outlined below:

35

-146-

10 pmoles of gene-specific primers  
10 pmoles of universal attB adapter-primers  
1 ng of plasmid containing the human hemoglobin cDNA.  
100 ng of human leukocyte cDNA library DNA.  
5  $\mu$ l of 10x PLATINUM Taq HiFi® reaction buffer (Life Technologies, Inc.)  
2  $\mu$ l of 50 mM MgSO<sub>4</sub>  
1  $\mu$ l of 10 mM dNTPs  
0.2  $\mu$ l of PLATINUM Taq HiFi® (1.0 unit)  
H<sub>2</sub>O to 50  $\mu$ l total reaction volume

Cycling conditions:

25 x 

95°C/5 min
94°C/15 sec
50°C/30 sec
68°C/1 min
68°C/5 min
5°C/hold

To assess the efficiency of the method, 2  $\mu$ l (1/25) of the 50  $\mu$ l PCR  
reaction was electrophoresed in a 3 % Agarose-1000 gel. With overlaps of 12 bp  
or less, smaller intermediate products containing one or no universal attB adapter  
predominated the reactions. Further optimization of PCR reaction conditions was  
obtained by titrating the amounts of gene-specific primers and universal attB  
adapter-primers. The PCR reactions were set up as outlined above except that the  
amounts of primers added were:

0, 1, 3 or 10 pmoles of gene-specific primers  
0, 10, 30 or 100 pmoles of adapter-primers

-147-

Cycling conditions:

25 x 

95°C/3 min
94°C/15 sec
50°C/45 sec
68°C/1 min
68°C/5 min
5°C/hold

The use of limiting amounts of gene-specific primers (3 pmoles) and excess adapter-primers (30 pmoles) reduced the amounts of smaller intermediate products. Using these reaction conditions the overlap necessary to obtain predominately full-length PCR product was reduced to 12 bp. The amounts of gene-specific and adapter-primers was further optimized in the following PCR reactions:

0, 1, 2 or 3 pmoles of gene-specific primers  
0, 30, 40 or 50 pmoles of adapter-primers

Cycling conditions:

25 x 

95°C/3 min
94°C/15 sec
48°C/1 min
68°C/1 min
68°C/5 min
5°C/hold

The use of 2 pmoles of gene-specific primers and 40 pmoles of adapter-primers further reduced the amounts of intermediate products and generated predominately full-length PCR products with gene-specific primers containing an 11 bp overlap. The success of the PCR reactions can be assessed in any PCR application by performing a no adapter control. The use of limiting amounts of gene-specific primers should give faint or barely visible bands when 1/25 to 1/10 of the PCR reaction is electrophoresed on a standard agarose gel. Addition of the

universal attB adapter-primers should generate a robust PCR reaction with a much higher overall yield of product.

PCR products from reactions using the 18 bp, 15 bp, 12 bp, 11 bp and 10 bp overlap gene-specific primers were purified using the CONCERT® Rapid PCR Purification System (PCR products greater than 500 bp can be PEG precipitated). The purified PCR products were subsequently cloned into an attP containing plasmid vector using the GATEWAY™ PCR Cloning System (Life Technologies, Inc.; Rockville, MD) and transformed into *E. coli*. Colonies were selected and counted on the appropriate antibiotic media and screened by PCR for correct inserts and orientation.

Raw PCR products (unpurified) from the attB adapter PCR of a plasmid clone of part of the human beta-globin (Hgb) gene were also used in GATEWAY™ PCR Cloning System reactions. PCR products generated with the full attB B1/B2-Hgb, the 12B1/B2, 11B1/B2 and 10B1/B2 attB overlap Hgb primers were successfully cloned into the GATEWAY™ pENTR21 attP vector (Figure 49). 24 colonies from each (24 x 4 = 96 total) were tested and each was verified by PCR to contain correct inserts. The cloning efficiency expressed as cfu/ml is shown below:

Primer Used	cfu/ml
Hgb full attB	8,700
Hgb 12 bp overlap	21,000
Hgb 11 bp overlap	20,500
Hgb 10 bp overlap	13,500
GFP control	1,300

Interestingly, the overlap PCR products cloned with higher efficiency than did the full attB PCR product. Presumably, and as verified by visualization on agarose gel, the adapter PCR products were slightly cleaner than was the full attB PCR product. The differences in colony output may also reflect the proportion of PCR product molecules with intact attB sites.

Using the attB adapter PCR method, PCR primers with 12 bp attB overlaps were used to amplify cDNAs of different sizes (ranging from 1 to 4 kb)

from a leukocyte cDNA library and from first strand cDNA prepared from HeLa total RNA. While three of the four cDNAs were able to be amplified by this method, a non-specific amplification product was also observed that under some conditions would interfere with the gene-specific amplification. This non-specific product was amplified in reactions containing the attB adapter-primers alone without any gene-specific overlap primers present. The non-specific amplification product was reduced by increasing the stringency of the PCR reaction and lowering the attB adapter PCR primer concentration.

These results indicate that the adapter-primer PCR approach described in this Example will work well for cloned genes. These results also demonstrate the development of a simple and efficient method to amplify PCR products that are compatible with the GATEWAY™ PCR Cloning System that allows the use of shorter gene-specific primers that partially overlap universal attB adapter-primers. In routine PCR cloning applications, the use of 12 bp overlaps is recommended. The methods described in this Example can thus reduce the length of gene-specific primers by up to 17 residues or more, resulting in a significant savings in oligonucleotide costs for high volume users of the GATEWAY™ PCR Cloning System. In addition, using the methods and assays described in this Example, one of ordinary skill can, using only routine experimentation, design and use analogous primer-adapters based on or containing other recombination sites or fragments thereof, such as attL, attR, attP, lox, FRT, etc.

***Example 21: Mutational Analysis of the Bacteriophage Lambda attL and attR Sites: Determinants of att Site Specificity in Site-specific Recombination***

To investigate the determinants of att site specificity, the bacteriophage lambda attL and attR sites were systematically mutagenized. As noted herein, the determinants of specificity have previously been localized to the 7 bp overlap region (TTTATAC, which is defined by the cut sites for the integrase protein and is the region where strand exchange takes place) within the 15 bp core region (GCTTTTTTTATACTAA) which is identical in all four lambda att sites, attB, attP, attL and attR. This core region, however, has not heretofore been systematically

mutagenized and examined to define precisely which mutations produce unique changes in *att* site specificity.

Therefore, to examine the effect of *att* sequence on site specificity, mutant *attL* and *attR* sites were generated by PCR and tested in an *in vitro* site-specific recombination assay. In this way all possible single base pair changes within the 7 bp overlap region of the core *att* site were generated as well as five additional changes outside the 7 bp overlap but within the 15 bp core *att* site. Each *attL* PCR substrate was tested in the *in vitro* recombination assay with each of the *attR* PCR substrates.

### Methods

To examine both the efficiency and specificity of recombination of mutant *attL* and *attR* sites, a simple *in vitro* site-specific recombination assay was developed. Since the core regions of *attL* and *attR* lie near the ends of these sites, it was possible to incorporate the desired nucleotide base changes within PCR primers and generate a series of PCR products containing mutant *attL* and *attR* sites. PCR products containing *attL* and *attR* sites were used as substrates in an *in vitro* reaction with GATEWAY™ LR Clonase™ Enzyme Mix (Life Technologies, Inc.; Rockville, MD). Recombination between a 1.5 kb *attL* PCR product and a 1.0 kb *attR* PCR product resulted in a 2.5 kb recombinant molecule that was monitored using agarose gel electrophoresis and ethidium bromide staining.

Plasmid templates pEZC1301 (Figure 84) and pEZC1313 (Figure 85), each containing a single wild type *attL* or *attR* site, respectively, were used for the generation of recombination substrates. The following list shows primers that were used in PCR reactions to generate the *attL* PCR products that were used as substrates in L x R Clonase reactions (capital letters represent changes from the wild-type sequence, and the underline represents the 7 bp overlap region within the 15 bp core *att* site; a similar set of PCR primers was used to prepare the *attR* PCR products containing matching mutations):

-151-

GATEWAY™ sites (note: attL2 sequence in GATEWAY™ plasmids begins "accca" while the attL2 site in this example begins "agcct" to reflect wild-type attL outside the core region.):

5

attL1: gggg agcct gcttttttGtacAaa gttggcatta taaaaa-  
agca ttgc

10

attL2: gggg agcct gcttttCttGtacAaa gttggcatta taaaaa-  
agca ttgc

Wild-type:

15

attL0: gggg agcct gctttttttatactaa gttggcatta taaaaa-  
agca ttgc

Single base changes from wild-type :

attLT1A: gggg agcct gcttttAttatactaa gttggcatta taaaaa-  
agca ttgc

20

attLT1C: gggg agcct gcttttCttatactaa gttggcatta taaaaa-  
agca ttgc

attLT1G: gggg agcct gcttttGttatactaa gttggcatta taaaaa-  
agca ttgc

25

attLT2A: gggg agcct gcttttAtatactaa gttggcatta taaaaa-  
agca ttgc

attLT2C: gggg agcct gcttttCtatactaa gttggcatta taaaaa-  
agca ttgc

30

attLT2G: gggg agcct gcttttGtatactaa gttggcatta taaaaa-  
aagca ttgc

35

-152-

attLT3A: gggg agcct gctttttAatactaa gttggcatta taaaa-  
aagca ttgc

5 attLT3C: gggg agcct gctttttCatactaa gttggcatta taaaa-  
aagca ttgc

10 attLT3G: gggg agcct gctttttGatactaa gttggcatta taaaa-  
aagca ttgc

15 attLA4C: gggg agcct gcttttttCtactaa gttggcatta taaaa-  
aagca ttgc

attLA4G: gggg agcct gcttttttGtactaa gttggcatta taaaa-  
aagca ttgc

20 attLA4T: gggg agcct gcttttttTtactaa gttggcatta taaaa-  
aagca ttgc

25 attLT5A: gggg agcct gcttttttaAactaa gttggcatta taaaa-  
aagca ttgc

attLT5C: gggg agcct gcttttttaCactaa gttggcatta taaaa-  
aagca ttgc

30 attLT5G: gggg agcct gcttttttaGactaa gttggcatta taaaa-  
aagca ttgc

35 attLA6C: gggg agcct gcttttttatCctaa gttggcatta taaaa-  
aagca ttgc



-153-

attLA6G: gggg agcct gcttttttatGctaa gttggcatta taaaa-  
aagca ttgc

5 attLA6T: gggg agcct gcttttttatTctaa gttggcatta taaaa-  
aagca ttgc

10 attLC7A: gggg agcct gcttttttataAtaa gttggcatta taaaa-  
aagca ttgc

15 attLC7G: gggg agcct gcttttttataGtaa gttggcatta taaaa-  
aagca ttgc

attLC7T: gggg agcct gcttttttataTtaa gttggcatta taaaa-  
aagca ttgc

Single base changes outside of the 7 bp overlap:

20 attL8: gggg agcct Acttttttataactaa gttggcatta taaaa-  
aagca ttgc

25 attL9: gggg agcct gcCtttttataactaa gttggcatta taaaaa-  
agca ttgc

attL10: gggg agcct gcttCtttataactaa gttggcatta taaaaa-  
agca ttgc

30 attL14: gggg agcct gcttttttataacCaa gttggcatta taaaaa-  
agca ttgc

35 attL15: gggg agcct gcttttttataactaG gttggcatta taaaaa-  
agca ttgc

-154-

Note: additional vectors wherein the first nine bases are gggg agcca (*i.e.*, substituting an adenine for the thymine in the position immediately preceding the 15-bp core region), which may or may not contain the single base pair substitutions (or deletions) outlined above, can also be used in these experiments.

5

Recombination reactions of *attL*- and *attR*-containing PCR products was performed as follows:

8 µl of H<sub>2</sub>O  
10 2 µl of attL PCR product (100 ng)  
2 µl of attR PCR product (100 ng)  
4 µl of 5x buffer  
4 µl of GATEWAY™ LR Clonase™ Enzyme Mix  
20 µl total volume

15

Clonase reactions were incubated at 25°C for 2 hours.

2 µl of 10X Clonase stop solution (proteinase K, 2 mg/ml) were added to stop the reaction.

10 µl were run on a 1 % agarose gel.

20

### Results

Each *attL* PCR substrate was tested in the *in vitro* recombination assay with each of the *attR* PCR substrates. Changes within the first three positions of the 7 bp overlap (TTTATAC) strongly altered the specificity of recombination.  
25 These mutant *att* sites each recombined as well as the wild-type, but only with their cognate partner mutant; they did not recombine detectably with any other *att* site mutant. In contrast, changes in the last four positions (TTTATAC) only partially altered specificity; these mutants recombined with their cognate mutant as well as wild-type *att* sites and recombined partially with all other mutant *att*  
30 sites except for those having mutations in the first three positions of the 7 bp

overlap. Changes outside of the 7 bp overlap were found not to affect specificity of recombination, but some did influence the efficiency of recombination.

Based on these results, the following rules for *att* site specificity were determined:

- Only changes within the 7 bp overlap affect specificity.
- Changes within the first 3 positions strongly affect specificity.
- Changes within the last 4 positions weakly affect specificity.

Mutations that affected the overall efficiency of the recombination reaction were also assessed by this method. In these experiments, a slightly increased (less than 2-fold) recombination efficiency with *attLT1A* and *attLC7T* substrates was observed when these substrates were reacted with their cognate *attR* partners. Also observed were mutations that decreased recombination efficiency (approximately 2-3 fold), including *attLA6G*, *attL14* and *attL15*. These mutations presumably reflect changes that affect Int protein binding at the core *att* site.

The results of these experiments demonstrate that changes within the first three positions of the 7 bp overlap (TTT*TATAC*) strongly altered the specificity of recombination (*i.e.*, *att* sequences with one or more mutations in the first three thymidines would only recombine with their cognate partners and would not cross-react with any other *att* site mutation). In contrast, mutations in the last four positions (*TTT**TATAC*) only partially altered specificity (*i.e.*, *att* sequences with one or more mutations in the last four base positions would cross-react partially with the wild-type *att* site and all other mutant *att* sites, except for those having mutations in one or more of the first three positions of the 7 bp overlap). Mutations outside of the 7 bp overlap were not found to affect specificity of recombination, but some were found to influence (*i.e.*, to cause a decrease in) the efficiency of recombination.

***Example 22: Discovery of Att Site Mutations That Increase the Cloning Efficiency of GATEWAY™ Cloning Reactions***

In experiments designed to understand the determinants of *att* site specificity, point mutations in the core region of *attL* were made. Nucleic acid molecules containing these mutated *attL* sequences were then reacted in an LR

reaction with nucleic acid molecules containing the cognate *attR* site (*i.e.*, an *attR* site containing a mutation corresponding to that in the *attL* site), and recombinational efficiency was determined as described above. Several mutations located in the core region of the att site were noted that either slightly increased (less than 2-fold) or decreased (between 2-4-fold) the efficiency of the recombination reaction (Table 3).

Table 3. *Effects of attL mutations on Recombination Reactions.*

Site	Sequence	Effect on Recombination
attL0	agcctgcttttttataactaagttggcatta	
attL5	agcctgcttttAttataactaagttggcatta	slightly increased
attL6	agcctgctttttttataTtaagttggcatta	slightly increased
attL13	agcctgctttttttatGctaagttggcatta	decreased
attL14	agcctgctttttttatacCaagttggcatta	decreased
attL15	agcctgctttttttataactaGgttggcatta	decreased
consensus	CAACTTnnTnnnAnnAAGTTG	

It was also noted that these mutations presumably reflected changes that either increased or decreased, respectively, the relative affinity of the integrase protein for binding the core att site. A consensus sequence for an integrase core-binding site (CAACTTNNT) has been inferred in the literature but not directly tested (see, *e.g.*, Ross and Landy, *Cell* 33:261-272 (1983)). This consensus core integrase-binding sequence was established by comparing the sequences of each of the four core att sites found in attP and attB as well as the sequences of five non-att sites that resemble the core sequence and to which integrase has been shown to bind in vitro. These experiments suggest that many more att site mutations might be identified which increase the binding of integrase to the core att site and thus increase the efficiency of GATEWAY™ cloning reactions.

**Example 23: Effects of Core Region Mutations on Recombination Efficiency**

To directly compare the cloning efficiency of mutations in the att site core region, single base changes were made in the attB2 site of an attB1-TET-attB2 PCR product. Nucleic acid molecules containing these mutated *attB2* sequences were then reacted in a BP reaction with nucleic acid molecules containing non-cognate *attP* sites (*i.e.*, wildtype *attP2*), and recombinational efficiency was determined as described above. The cloning efficiency of these mutant attB2 containing PCR products compared to standard attB1-TET-attB2 PCR product are shown in Table 4.

Table 4. Efficiency of Recombination With Mutated attB2 Sites.

	<u>Site</u>	<u>Sequence</u>	<u>Mutation</u>	<u>Cloning Efficiency</u>
15	attB0	tcaagttagtataaaaaagcaggct		
	attB1	ggggacaagtttgtacaaaaagcaggct		
	attB2	ggggaccactttgtacaagaaagctgggt		100%
	attB2.1	ggggaAcactttgtacaagaaagctgggt	C→A	40%
	attB2.2	ggggacAactttgtacaagaaagctgggt	C→A	131%
20	attB2.3	ggggaccCctttgtacaagaaagctgggt	A→C	4%
	attB2.4	ggggaccaAttgtacaagaaagctgggt	C→A	11%
	attB2.5	ggggaccacGttgtacaagaaagctgggt	T→G	4%
	attB2.6	ggggaccactGtgtacaagaaagctgggt	T→G	6%
	attB2.7	ggggaccacttGgtacaagaaagctgggt	T→G	1%
25	attB2.8	ggggaccactttTtacaagaaagctgggt	G→T	0.5%

As noted above, a single base change in the attB2.2 site increased the cloning efficiency of the attB1-TET-attB2.2 PCR product to 131% compared to the attB1-TET-attB2 PCR product. Interestingly, this mutation changes the integrase core binding site of attB2 to a sequence that matches more closely the proposed consensus sequence.

Additional experiments were performed to directly compare the cloning efficiency of an attB1-TET-attB2 PCR product with a PCR product that contained attB sites containing the proposed consensus sequence (*see* Example 22) of an integrase core binding site. The following attB sites were used to amplify attB-TET PCR products:

```
attB1      ggggacaagtttgtacaaaaaagcaggct
attB1.6    ggggacaaCtttgtacaaaaaagTTggct
attB2      ggggaccactttgtacaagaaagctgggt
attB2.10   ggggacAactttgtacaagaaagTtgggt
```

BP reactions were carried out between 300 ng (100 fmoles) of pDONR201 (Figure 49A) with 80 ng (80 fmoles) of attB-TET PCR product in a 20  $\mu$ l volume with incubation for 1.5 hrs at 25°C, creating pENTR201-TET Entry clones. A comparison of the cloning efficiencies of the above-noted attB sites in BP reactions is shown in Table 5.

*Table 5. Cloning efficiency of BP Reactions.*

PCR product	CFU/ml	Fold Increase
B1-tet-B2	7,500	
B1.6-tet-B2	12,000	1.6 x
B1-tet-B2.10	20,900	2.8 x
B1.6-tet-B2.10	30,100	4.0 x

These results demonstrate that attB PCR products containing sequences that perfectly match the proposed consensus sequence for integrase core binding sites can produce Entry clones with four-fold higher efficiency than standard Gateway attB1 and attB2 PCR products.

The entry clones produced above were then transferred to pDEST20 (Figure 40A) via LR reactions (300 ng (64 fmoles) pDEST20 mixed with 50 ng (77 fmoles) of the respective pENTR201-TET Entry clone in 20  $\mu$ l volume; incubated for 1 hr incubation at 25°C). The efficiencies of cloning for these reactions are compared in Table 6.

Table 6. Cloning Efficiency of LR Reactions.

pENTR201-TET x pDEST20	CFU/ml	Fold Increase
L1-tet-L2	5,800	
L1.6-tet-L2	8,000	1.4
L1-tet-L2.10	10,000	1.7
L1.6-tet-L2.10	9,300	1.6

These results demonstrate that the mutations introduced into attB1.6 and attB2.10 that transfer with the gene into entry clones slightly increase the efficiency of LR reactions. Thus, the present invention encompasses not only mutations in *attB* sites that increase recombination efficiency, but also to the corresponding mutations that result in the *attL* sites created by the BP reaction.

To examine the increased cloning efficiency of the attB1.6-TET-attB2.10 PCR product over a range of PCR product amounts, experiments analogous to those described above were performed in which the amount of attB PCR product was titrated into the reaction mixture. The results are shown in Table 7.

Table 7. Titration of attB PCR products.

Amount of attB PCR product (ng)	PCR product	CFU/ml	Fold Increase
20	attB1-TET-attB2	3,500	6.1
	attB1.6-TET-attB2.10	21,500	
50	attB1-TET-attB2	9,800	5.0
	attB1.6-TET-attB2.10	49,000	
100	attB1-TET-attB2	18,800	2.8
	attB1.6-TET-attB2.10	53,000	
200	attB1-TET-attB2	19,000	2.5
	attB1.6-TET-attB2.10	48,000	

These results demonstrate that as much as a six-fold increase in cloning efficiency is achieved with the attB1.6-TET-attB2.10 PCR product as compared to the standard attB1-TET-attB2 PCR product at the 20 ng amount.

**Example 24: Determination of attB Sequence Requirements for Optimum Recombination Efficiency**

To examine the sequence requirements for attB and to determine which attB sites would clone with the highest efficiency from populations of degenerate attB sites, a series of experiments was performed. Degenerate PCR primers were designed which contained five bases of degeneracy in the B-arm of the attB site. These degenerate sequences would thus transfer with the gene into Entry clone in BP reactions and subsequently be transferred with the gene into expression clones in LR reactions. The populations of degenerate attB and attL sites could thus be cycled from attB to attL back and forth for any number of cycles. By altering the reaction conditions at each transfer step (for example by decreasing the reaction time and/or decreasing the concentration of DNA) the reaction can be made increasingly more stringent at each cycle and thus enrich for populations of attB and attL sites that react more efficiently.

The following degenerate PCR primers were used to amplify a 500 bp fragment from pUC18 which contained the lacZ alpha fragment (only the attB portion of each primer is shown):

```

attB1      GGGG ACAAGTTTGTACAAA AAAGC AGGCT
attB1n16-20 GGGG ACAAGTTTGTACAAA nnnnn AGGCT
attB1n21-25 GGGG ACAAGTTTGTACAAA AAAGC nnnnn

attB2      GGGG ACCACTTTGTACAAG AAAGC TGGGT
attB2n16-20 GGGG ACCACTTTGTACAAG nnnnn TGGGT
attB2n21-25 GGGG ACCACTTTGTACAAG AAAGC nnnnn

```

The starting population size of degenerate att sites is  $4^5$  or 1024 molecules. Four different populations were transferred through two BP reactions and two LR reactions. Following transformation of each reaction, the population of transformants was amplified by growth in liquid media containing the appropriate selection antibiotic. DNA was prepared from the population of clones by alkaline



-161-

lysis miniprep and used in the next reaction. The results of the BP and LR cloning reactions are shown below.

## BP-1, overnight reactions

	cfu/ml	percent of control
attB1-LacZa-attB2	78,500	100 %
attB1n16-20-LacZa-attB2	1,140	1.5 %
attB1n21-25-LacZa-attB2	11,100	14 %
attB1-LacZa-attB2n16-20	710	0.9 %
attB1-LacZa-attB2n21-25	16,600	21 %

LR-1, pENTR201-LacZa x pDEST20/*EcoRI*, 1hr reactions

	cfu/ml	percent of control
attL1-LacZa-attL2	20,000	100 %
attL1n16-20-LacZa-attL2	2,125	11 %
attL1n21-25-LacZa-attL2	2,920	15 %
attL1-LacZa-attL2n16-20	3,190	16 %
attL1-LacZa-attL2n21-25	1,405	7 %

BP-2, pEXP20-LacZa/*ScaI* x pDONR 201, 1hr reactions

	cfu/ml	percent of control
attB1-LacZa-attB2	48,600	100 %
attB1n16-20-LacZa-attB2	22,800	47 %
attB1n21-25-LacZa-attB2	31,500	65 %
attB1-LacZa-attB2n16-20	42,400	87 %
attB1-LacZa-attB2n21-25	34,500	71 %

LR-2, pENTR201-LacZa x pDEST6/*NcoI*, 1hr reactions

	cfu/ml	percent of control
attL1-LacZa-attL2	23,000	100 %
attL1n16-20-LacZa-attL2	49,000	213 %
attL1n21-25-LacZa-attL2	18,000	80 %
attL1-LacZa-attL2n16-20	37,000	160 %
attL1-LacZa-attL2n21-25	57,000	250 %

These results demonstrate that at each successive transfer, the cloning efficiency of the entire population of att sites increases, and that there is a great deal of flexibility in the definition of an attB site. Specific clones may be isolated from the above reactions, tested individually for recombination efficiency, and

sequenced. Such new specificities may then be compared to known examples to guide the design of new sequences with new recombination specificities. In addition, based on the enrichment and screening protocols described herein, one of ordinary skill can easily identify and use sequences in other recombination sites, *e.g.*, other *att* sites, *lox*, FRT, etc., that result in increased specificity in the recombination reactions using nucleic acid molecules containing such sequences.

***Example 25: Design of att Site PCR Adapter-Primers***

Additional studies were performed to design gene-specific primers with 12bp of attB1 and attB2 at their 5'-ends. The optimal primer design for *att*-containing primers is the same as for any PCR primers: the gene-specific portion of the primers should ideally have a  $T_m$  of  $> 50^\circ\text{C}$  at 50 mM salt (calculation of  $T_m$  is based on the formula  $59.9 + 41(\%GC) - 675/n$ ).

Primers:

12bp attB1: AA AAA GCA GGC TNN - forward gene-specific primer

12bp attB2: A GAA AGC TGG GTN - reverse gene-specific primer

attB1 adapter primer: GGGGACAAGTTTGTACAAAAAAGCAGGCT

attB2 adapter primer: GGGGACCACTTTGTACAAGAAAGCTGGGT

Protocol:

(1) Mix 200 ng of cDNA library or 1 ng of plasmid clone DNA (alternatively, genomic DNA or RNA could be used) with 10 pmoles of gene specific primers in a 50  $\mu\text{l}$  PCR reaction, using one or more polypeptides having DNA polymerase activity such as those described herein. (The addition of greater than 10 pmoles of gene-specific primers can decrease the yield of attB PCR product. In addition, if RNA is used, a standard reverse transcriptase-PCR (RT-

PCR) protocol should be followed; *see, e.g.,* Gerard, G.F., *et al.*, *FOCUS* 11:60 (1989); Myers, T.W., and Gelfand, D.H., *Biochem.* 30:7661 (1991); Freeman, W.N., *et al.*, *BioTechniques* 20:782 (1996); and U.S. Application No. 09/064,057, filed April 22, 1998, the disclosures of all of which are incorporated herein by reference.)

1<sup>st</sup> PCR profile:

(a) 95°C for 3 minutes

(b) 10 cycles of:

(i) 94°C for 15 seconds

(ii) 50°C\* for 30 seconds

(iii) 68°C for 1 minute/kb of target amplicon

(c) 68°C for 5 minutes

(d) 10°C hold

\*The optimal annealing temperature is determined by the calculated T<sub>m</sub> of the gene-specific part of the primer.

(2) Transfer 10 µl to a 40 µl PCR reaction mix containing 35 pmoles each of the attB1 and attB2 adapter primers.

2<sup>nd</sup> PCR profile:

(a) 95°C for 1 minute

(b) 5 cycles of:

(i) 94°C for 15 seconds

(ii) 45°C\* for 30 seconds

(iii) 68°C for 1 minute/kb of target amplicon

(c) 15-20 cycles\*\* of:

(i) 94°C for 15 seconds

(ii) 55°C\* for 30 seconds

-164-

- (iii) 68°C for 1 minute/kb of target amplicon
- (d) 68°C for 5 minutes
- (e) 10°C hold

5        \*The optimal annealing temperature is determined by the calculated  $T_m$  of the gene-specific part of the primer.

      \*\*15 cycles is sufficient for low complexity targets.

Notes:

- 10        1.        It is useful to perform a no-adapter primer control to assess the yield of attB PCR product produced.
2.        Linearized template usually results in slightly greater yield of PCR product.

15

***Example 26: One-Tube Recombinational Cloning Using the GATEWAY™ Cloning System***

20        To provide for easier and more rapid cloning using the GATEWAY™ cloning system, we have designed a protocol whereby the BP and LR reactions may be performed in a single tube (a "one-tube" protocol). The following is an example of such a one-tube protocol; in this example, an aliquot of the BP reaction is taken before adding the LR components, but the BP and LR reactions may be performed in a one-tube protocol without first taking the BP aliquot:

25

<u>Reaction Component</u>	<u>Volume</u>
attB DNA (100-200 ng/25 µl reaction)	1-12.5 µl
attP DNA (pDONR201) 150 ng/µl	2.5 µl
5X BP Reaction Buffer	5.0 µl
30        Tris-EDTA	(to 20 µl)
<u>BP Clonase</u>	<u>5.0 µl</u>
Total vol.	25 µl

-165-

After the above components were mixed in a single tube, the reaction mixtures were incubated for 4 hours at 25°C. A 5 µl aliquot of reaction mixture was removed, and 0.5 µl of 10X stop solution was added to this reaction mixture and incubated for 10 minutes at 37°C. Competent cells were then transformed with 1-2 µl of the BP reaction per 100 µl of cells; this transformation yielded colonies of Entry Clones for isolation of individual Entry Clones and for quantitation of the BP Reaction efficiency.

To the remaining 20 µl of BP reaction mixture, the following components of the LR reaction were added:

<u>Reaction Component</u>	<u>Final Concentration</u>	<u>Volume Added</u>
NaCl	0.75 M	1 µl
Destination Vector	150 ng/ul	3 µl
<u>LR Clonase</u>		<u>6 µl</u>
Total vol.		30 µl

After the above components were mixed in a single tube, the reaction mixtures were incubated for 2 hours at 25°C. 3 µl of 10X stop solution was added, and the mixture was incubated for 10 minutes at 37°C. Competent cells were then transformed with 1-2 µl of the reaction mixture per 100 µl of cells

Notes:

1. If desired, the Destination Vector can be added to the initial BP reaction.
2. The reactions can be scaled down by 2x, if desired.
3. Shorter incubation times for the BP and/or LR reactions can be used (scaled to the desired cloning efficiencies of the reaction), but a lower number of colonies will typically result.
4. To increase the number of colonies obtained by several fold, incubate the BP reaction for 6-20 hours and increase the LR reaction to 3 hours. Electroporation also works well with 1-2 ul of the PK-treated reaction mixture.

5. PCR products greater than about 5 kb may show significantly lower cloning efficiency in the BP reaction. In this case, we recommend using a one-tube reaction with longer incubation times (*e.g.*, 6-18 hours) for both the BP and LR steps.

5

***Example 27: Relaxation of Destination Vectors During the LR Reaction***

To further optimize the LR Reaction, the composition of the LR Reaction buffer was modified from that described above and this modified buffer was used in a protocol to examine the impact of enzymatic relaxation of Destination Vectors during the LR Reaction.

10

LR Reactions were set up as usual (*see, e.g.*, Example 6), except that 5X BP Reaction Buffer (*see* Example 5) was used for the LR Reaction. To accomplish Destination Vector relaxation during the LR Reaction, Topoisomerase I (Life Technologies, Inc., Rockville, MD; Catalogue No. 38042-016) was added to the reaction mixture at a final concentration of ~15U per  $\mu\text{g}$  of total DNA in the reaction (for example, for reaction mixtures with a total of 400ng DNA in the 20  $\mu\text{l}$  LR Reaction, ~6units of Topoisomerase I was added). Reaction mixtures were set up as follows:

15

20

<u>Reaction Component</u>	<u>Volume</u>
ddH <sub>2</sub> O	6.5 $\mu\text{l}$
4X BP Reaction Buffer	5 $\mu\text{l}$
100ng single chain/linear pENTR CAT, 50 ng/ $\mu\text{l}$	2 $\mu\text{l}$
300ng single chain/linear pDEST6, 150ng/ $\mu\text{l}$	2 $\mu\text{l}$
Topoisomerase I, 15 U/ml	0.5 $\mu\text{l}$
LR Clonase	4 $\mu\text{l}$

25

Reaction mixtures were incubated at 25°C for 1 hour, and 2  $\mu\text{l}$  of 2  $\mu\text{g}/\mu\text{l}$  Proteinase K was then added and mixtures incubated for 10 minutes at 37°C to stop the LR Reaction. Competent cells were then transformed as described in the preceding examples. The results of these studies demonstrated that relaxation of

30

substrates in the LR reaction using Topoisomerase I resulted in a 2- to 10-fold increase in colony output compared to those LR reactions performed without including Topoisomerase I.

5           Having now fully described the present invention in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious to one of ordinary skill in the art that the same can be performed by modifying or changing the invention within a wide and equivalent range of conditions, formulations and other parameters without affecting the scope of the invention or  
10           any specific embodiment thereof, and that such modifications or changes are intended to be encompassed within the scope of the appended claims.

          All publications, patents and patent applications mentioned in this specification are indicative of the level of skill of those skilled in the art to which this invention pertains, and are herein incorporated by reference to the same extent  
15           as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference.

167.1

Applicant's or agent's file reference number	0942.558PC03	International application No. 11	PCT/US 00/05432
--	--------------	----------------------------------	-----------------

**INDICATIONS RELATING TO DEPOSITED MICROORGANISM  
OR OTHER BIOLOGICAL MATERIAL**  
(PCT Rule 13bis)

REC'D 17 APR 2000

WIPO PCT

A. The indications made below relate to the microorganism referred to in the description on page 52, line 31.

**B. IDENTIFICATION OF DEPOSIT**Further deposits are identified on an additional sheet ☒

Name of depositary institution  
Agricultural Research Culture Collection (NRRL)  
International Depository Authority

Address of depositary institution (including postal code and country)

1815 N. University Street  
Peoria, Illinois 61604  
United States of America

Date of deposit  
February 27, 1999

Accession Number  
NRRL B-30099

**C. ADDITIONAL INDICATIONS** (leave blank if not applicable)This information is continued on an additional sheet ☐

Escherichia coli DB3.1(pAHPKan) or Escherichia coli DB3.1(pAttPKan)

**D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE** (if the indications are not for all designated States)**E. SEPARATE FURNISHING OF INDICATIONS** (leave blank if not applicable)

The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")

For receiving Office use only		For International Bureau use only	
<input checked="" type="checkbox"/> This sheet was received with the international application		<input type="checkbox"/> This sheet was received by the International Bureau on:	
Authorized officer Date of receipt: <u>17 APR 2000</u> Signature: <u>[Signature]</u> Stamp: <u>NRRL B-30099 (F)</u>		Authorized officer Date of receipt: <u>17 APR 2000</u> Signature: <u>[Signature]</u> Stamp: <u>NRRL B-30099 (F)</u>	



Applicant's or agent's file reference number	0942.468PC03	167.2 International application No. tl <b>PCT/US 00/05432</b>
---	--------------	---

**INDICATIONS RELATING TO DEPOSITED MICROORGANISM  
OR OTHER BIOLOGICAL MATERIAL**  
(PCT Rule 13bis)

REC'D 17 APR 2000

WIPO PCT

A. The indications made below relate to the microorganism referred to in the description on page 16, line 55.

**B. IDENTIFICATION OF DEPOSIT**Further deposits are identified on an additional sheet ☒

Name of depository institution  
Agricultural Research Culture Collection (NRRL)  
International Depository Authority

Address of depository institution (including postal code and country)

1815 N. University Street  
Peoria, Illinois 61604  
United States of America

Date of deposit  
February 27, 1999

Accession Number  
NRRL B-30100

**C. ADDITIONAL INDICATIONS** (leave blank if not applicable)This information is continued on an additional sheet ☐

Escherichia coli DB3.1(pENTR-1A)

**D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE** (if the indications are not for all designated States)**E. SEPARATE FURNISHING OF INDICATIONS** (leave blank if not applicable)

The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")

For receiving Office use only	For International Bureau use only
<input checked="" type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer Barbara Fridle 100 CENTER STREET PEORIA, ILLINOIS 61604 UNITED STATES OF AMERICA	Authorized officer

Applicant's or agent's file reference number	International application No. tb
0942.468PC03	00/05432

**INDICATIONS RELATING TO DEPOSITED MICROORGANISM  
OR OTHER BIOLOGICAL MATERIAL**  
(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page 16 line 16.

### B. IDENTIFICATION OF DEPOSIT

Further deposits are identified on an additional sheet ☒

Name of depositary institution  
Agricultural Research Culture Collection (NRRL)  
International Depository Authority

Address of depositary institution (including postal code and country)

1815 N. University Street  
Peoria, Illinois 61604  
United States of America

Date of deposit  
February 27, 1999

Accession Number  
NRRL B-30101

**C. ADDITIONAL INDICATIONS** *(leave blank if not applicable)*

This information is continued on an additional sheet ☐

**Escherichia coli DB3.1(pENTR-2B)**

## D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)

**E. SEPARATE FURNISHING OF INDICATIONS** *(leave blank if not applicable)*

The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposits")

For receiving Office use only	For International Bureau use only
<input checked="" type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer Barbara Fricke WI Copyright Office 1000 N. 17th St., 10th Floor Arlington, VA 22209-4330 (E)	Authorized officer

167.4

Applicant's or agent's file reference number	0942.468PC03	International Application No. <b>PCT/US 01/05432</b>
INDICATIONS RELATING TO DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL (PCT Rule 13bis)		REC'D 17 APR 2000 ANISM
		WIPO PCT

A. The indications made below relate to the microorganism referred to in the description on page 55, line 16.

**B. IDENTIFICATION OF DEPOSIT**Further deposits are identified on an additional sheet ☒

Name of depositary institution  
Agricultural Research Culture Collection (NRRL)  
International Depositary Authority

Address of depositary institution (including postal code and country)

1815 N. University Street  
Peoria, Illinois 61604  
United States of America

Date of deposit  
February 27, 1999

Accession Number  
NRRL B-30102

**C. ADDITIONAL INDICATIONS** (leave blank if not applicable)This information is continued on an additional sheet ☐

Escherichia coli DB3.1(pENTR-3C)

**D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE** (if the indications are not for all designated States)**E. SEPARATE FURNISHING OF INDICATIONS** (leave blank if not applicable)

The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")

For receiving Office use only	For International Bureau use only
<input checked="" type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer <i>[Signature]</i> 155-5220 (F)	Authorized officer

167.5

Applicant's or agent's file reference number	0942.468PC03	International application No. tb	PCT/US 00/05432
--	--------------	----------------------------------	-----------------

**INDICATIONS RELATING TO DEPOSITED MICROORGANISM  
OR OTHER BIOLOGICAL MATERIAL**  
(PCT Rule 13bis)

RECEIVED	17
APR	2000
WIPO	PCT

A. The indications made below relate to the microorganism referred to in the description on page 8.	RECEIVED 17 APR 2000
	WIPO PCT

**B. IDENTIFICATION OF DEPOSIT**Further deposits are identified on an additional sheet ☒

Name of depositary institution  
Agricultural Research Culture Collection (NRRL)  
International Depositary Authority

Address of depositary institution (including postal code and country)

1815 N. University Street  
Peoria, Illinois 61604  
United States of America

Date of deposit  
February 27, 1999

Accession Number  
NRRL B-30103

**C. ADDITIONAL INDICATIONS** (leave blank if not applicable)This information is continued on an additional sheet ☐

Escherichia coli DB3.1(pEZC15101)

**D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE** (if the indications are not for all designated States)**E. SEPARATE FURNISHING OF INDICATIONS** (leave blank if not applicable)

The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")

For receiving Office use only	For International Bureau use only
<input checked="" type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

167.6

Applicant's or agent's file reference number	0942.468PC03	International application No. 1.	PCT/US 00/05432
---	--------------	----------------------------------	-----------------

INDICATIONS RELATING TO DEPOSITED MICROORGANISM  
OR OTHER BIOLOGICAL MATERIAL (PCT Rule 13bis)

REC'D 17

WFO

A. The indications made below relate to the microorganism referred to in the description on page <u>54</u> , line <u>9</u> .
--

B. IDENTIFICATION OF DEPOSIT	Further deposits are identified on an additional sheet <input checked="" type="checkbox"/>
------------------------------	--

Name of depositary institution  
Agricultural Research Culture Collection (NRRL)  
International Depository Authority

Address of depositary institution (including postal code and country)

1815 N. University Street  
Peoria, Illinois 61604  
United States of America

Date of deposit  
February 27, 1999

Accession Number  
NRRL B-30104

C. ADDITIONAL INDICATIONS (leave blank if not applicable)	This information is continued on an additional sheet <input type="checkbox"/>
---	---

Escherichia coli DB3.1(pEZC15102)

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)
--

E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)
---

The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")

For receiving Office use only	For International Bureau use only
<input checked="" type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

167.7

Applicant's or agent's file reference number	0942.468PC03	International application No. <b>PCT/US</b>	<b>00/05432</b>
--	--------------	---	-----------------

**INDICATIONS RELATING TO DEPOSITED MICROORGANISM  
OR OTHER BIOLOGICAL MATERIAL**  
(PCT Rule 13bis)

RECEIVED 17 APR 2000

V. T

A. The indications made below relate to the microorganism referred to in the description on page 54, line 9.

<b>B. IDENTIFICATION OF DEPOSIT</b>		Further deposits are identified on an additional sheet <input checked="" type="checkbox"/>
Name of depositary institution Agricultural Research Culture Collection (NRRL) International Depositary Authority		
Address of depositary institution (including postal code and country)  1815 N. University Street Peoria, Illinois 61604 United States of America		
Date of deposit February 27, 1999	Accession Number NRRL B-30105	

C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet ☐

Escherichia coli DB3.1(pEZC15103)

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)

E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)

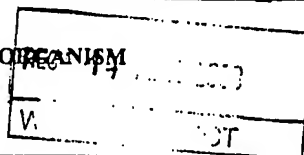
The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")

For receiving Office use only	For International Bureau use only
<input checked="" type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer <i>[Signature]</i>	Authorized officer

167.8

Applicant's or agent's file reference number	0942.408PC03	International application No. 11	PCT/US 00/05432
--	--------------	----------------------------------	-----------------

**INDICATIONS RELATING TO DEPOSITED MICROORGANISM  
OR OTHER BIOLOGICAL MATERIAL**  
(PCT Rule 13bis)



A. The indications made below relate to the microorganism referred to in the description on page <u>51</u> , line <u>20-21</u> .	
<b>B. IDENTIFICATION OF DEPOSIT</b> Further deposits are identified on an additional sheet <input checked="" type="checkbox"/>	
Name of depositary institution Agricultural Research Culture Collection (NRRL) International Depositary Authority	
Address of depositary institution (including postal code and country)  1815 N. University Street Peoria, Illinois 61604 United States of America	
Date of deposit February 27, 1999	Accession Number NRRL B-30108
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
Escherichia coli DB10B(pCMVSPORT6)	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indications are not for all designated States)	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")	

For receiving Office use only	For International Bureau use only
<input checked="" type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer Barbara Fricke BT	Authorized officer

## WHAT IS CLAIMED IS:

1. An isolated nucleic acid molecule comprising a nucleotide sequence selected from the group of nucleotide sequences consisting of an attB1 nucleotide sequence as set forth in Figure 9, an attB2 nucleotide sequence as set forth in Figure 9, an attP1 nucleotide sequence as set forth in Figure 9, an attP2 nucleotide sequence as set forth in Figure 9, an attL1 nucleotide sequence as set forth in Figure 9, an attL2 nucleotide sequence as set forth in Figure 9, an attR1 nucleotide sequence as set forth in Figure 9, an attR2 nucleotide sequence as set forth in Figure 9, a polynucleotide complementary thereto, and a mutant, fragment, or derivative thereof.

2. An isolated nucleic acid molecule comprising an attB1 nucleotide sequence as set forth in Figure 9, a polynucleotide complementary thereto, or a mutant, fragment, variant or derivative thereof.

3. An isolated nucleic acid molecule comprising an attB2 nucleotide sequence as set forth in Figure 9, a polynucleotide complementary thereto, or a mutant, fragment, variant or derivative thereof.

4. An isolated nucleic acid molecule comprising an attP1 nucleotide sequence as set forth in Figure 9, a polynucleotide complementary thereto, or a mutant, fragment, variant or derivative thereof.

5. An isolated nucleic acid molecule comprising an attP2 nucleotide sequence as set forth in Figure 9, a polynucleotide complementary thereto, or a mutant, fragment, variant or derivative thereof.

6. An isolated nucleic acid molecule comprising an attL1 nucleotide sequence as set forth in Figure 9, a polynucleotide complementary thereto, or a mutant, fragment, variant or derivative thereof.



7. An isolated nucleic acid molecule comprising an attL2 nucleotide sequence as set forth in Figure 9, a polynucleotide complementary thereto, or a mutant, fragment, variant or derivative thereof.

5 8. An isolated nucleic acid molecule comprising an attR1 nucleotide sequence as set forth in Figure 9, a polynucleotide complementary thereto, or a mutant, fragment, variant or derivative thereof.

10 9. An isolated nucleic acid molecule comprising an attR2 nucleotide sequence as set forth in Figure 9, a polynucleotide complementary thereto, or a mutant, fragment, variant or derivative thereof.

15 10. The isolated nucleic acid molecule of claim 1, further comprising one or more functional or structural nucleotide sequences selected from the group consisting of one or more multiple cloning sites, one or more localization signals, one or more transcription termination sites, one or more transcriptional regulatory sequences, one or more translational signals, one or more origins of replication, one or more fusion partner peptide-encoding nucleic acid molecules, one or more protease cleavage sites, and one or more 5' polynucleotide extensions.

20 11. The nucleic acid molecule of claim 10, wherein said transcriptional regulatory sequence is a promoter, an enhancer, or a repressor.

25 12. The nucleic acid molecule of claim 10, wherein said fusion partner peptide-encoding nucleic acid molecule encodes glutathione S-transferase (GST), hexahistidine (His<sub>6</sub>), or thioredoxin (Trx).

30 13. The nucleic acid molecule of claim 10, wherein said 5' polynucleotide extension consists of from one to five nucleotide bases.

14. The nucleic acid molecule of claim 13, wherein said 5' polynucleotide extension consists of four or five guanine nucleotide bases.

15. A primer nucleic acid molecule suitable for amplifying a target nucleotide sequence, comprising the isolated nucleic acid molecule of claim 1 or a portion thereof linked to a target-specific nucleotide sequence useful in amplifying said target nucleotide sequence.

16. The primer nucleic acid molecule of claim 15, wherein said primer comprises an attB1 nucleotide sequence having the sequence shown in Figure 9 or a portion thereof, or a polynucleotide complementary to the sequence shown in Figure 9 or a portion thereof.

17. The primer nucleic acid molecule of claim 15, wherein said primer comprises an attB2 nucleotide sequence having the sequence shown in Figure 9 or a portion thereof, or a polynucleotide complementary to the sequence shown in Figure 9 or a portion thereof.

18. The primer nucleic acid molecule of claim 15, further comprising a 5' terminal extension of four or five guanine bases.

19. A vector comprising the isolated nucleic acid molecule of claim 1.

20. The vector of claim 19, wherein said vector is an Expression Vector.

21. A host cell comprising the isolated nucleic acid molecule of claim 1 or the vector of claim 19.

22. A method of synthesizing or amplifying one or more nucleic acid molecules comprising:

- (a) mixing one or more nucleic acid templates with at least one polypeptide having polymerase or reverse transcriptase activity and at least a first primer comprising a template-specific sequence that is complementary to or capable of hybridizing to said

templates and at least a second primer comprising all or a portion of a recombination site wherein said at least a portion of said second primer is homologous to or complementary to at least a portion of said first primer; and

- 5 (b) incubating said mixture under conditions sufficient to synthesize or amplify one or more nucleic acid molecules complementary to all or a portion of said templates and comprising one or more recombination sites or portions thereof at one or both termini of said molecules.

10 23. A method of synthesizing or amplifying one or more nucleic acid molecules comprising:

- 15 (a) mixing one or more nucleic acid templates with at least one polypeptide having polymerase or reverse transcriptase activity and at least a first primer comprising a template-specific sequence that is complementary to or capable of hybridizing to said templates and at least a portion of a recombination site, and at least a second primer comprising all or a portion of a recombination site wherein said at least a portion of said recombination site on said second primer is complementary to or homologous to at least a portion of said recombination site on said first primer; and

- 20 (b) incubating said mixture under conditions sufficient to synthesize or amplify one or more nucleic acid molecules complementary to all or a portion of said templates and comprising one or more recombination sites or portions thereof at one or both termini of said molecules.

25 24. A method of amplifying or synthesizing one or more nucleic acid molecules comprising:

- 30 (a) mixing one or more nucleic acid templates with at least one polypeptide having polymerase or reverse transcriptase activity

and one or more first primers comprising at least a portion of a recombination site and a template-specific sequence that is complementary to or capable of hybridizing to said template;

(b) incubating said mixture under conditions sufficient to synthesize or amplify one or more first nucleic acid molecules complementary to all or a portion of said templates wherein said molecules comprise at least a portion of a recombination site at one or both termini of said molecules;

(c) mixing said molecules with one or more second primers comprising one or more recombination sites, wherein said recombination sites of said second primers are homologous to or complementary to at least a portion of said recombination sites on said first nucleic acid molecules; and

(d) incubating said mixture under conditions sufficient to synthesize or amplify one or more second nucleic acid molecules complementary to all or a portion of said first nucleic acid molecules and which comprise one or more recombination sites at one or both termini of said molecules.

25. A polypeptide encoded by the isolated nucleic acid molecule of any one of claims 1-10.

26. An isolated nucleic acid molecule comprising one or more *att* recombination sites comprising at least one mutation in its core region that increases the specificity of interaction between said recombination site and a second *att* recombination site.

27. The isolated nucleic acid molecule of claim 26, wherein said mutation is at least one substitution mutation of at least one nucleotide in the seven basepair overlap region of said core region of said recombination site.

28. The isolated nucleic acid molecule of claim 26, wherein said nucleic acid molecule comprises the sequence NNNATAC, wherein "N" refers to any nucleotide with the proviso that if one of the first three nucleotides in the consensus sequence is a T/U, then at least one of the other two of the first three nucleotides is not a T/U.

29. An isolated nucleic acid molecule comprising one or more mutated *att* recombination sites comprising at least one mutation in its core region that enhances the efficiency of recombination between a first nucleic acid molecule comprising said mutated *att* recombination site and a second nucleic acid molecule comprising a second recombination site that interacts with said mutated *att* recombination site.

30. The isolated nucleic acid molecule of claim 29, wherein said mutated *att* recombination site is a mutated *attL* site comprising a core region having the nucleotide sequence caactnntnnnannaagttg, wherein "n" represents any nucleotide.

31. The isolated nucleic acid molecule of claim 30, wherein said mutated *attL* recombination site comprises a core region having a nucleotide sequence selected from agcctgctttattataactaagttggcatta (*attL5*) and agcctgctttttatattaagttggcatta (*attL6*).

32. The isolated nucleic acid molecule of claim 29, wherein said mutated *att* recombination site comprises a core region having a nucleotide sequence selected from the group consisting of ggggacaactttgtacaaaaagttggct (*attB1.6*), ggggacaactttgtacaagaaagctgggt (*attB2.2*), and ggggacaactttgtacaagaaagttgggt (*attB2.10*).

33. A vector selected from the group consisting of pENTR1A, pENTR2B, pENTR3C, pENTR4, pENTR5, pENTR6, pENTR7, pENTR8, pENTR9, pENTR10, pENTR11, pDEST1, pDEST2, pDEST3, pDEST4,

pDEST5, pDEST6, pDEST7, pDEST8, pDEST9, pDEST10, pDEST11, pDEST12.2 (also known as pDEST12), pDEST13, pDEST14, pDEST15, pDEST16, pDEST17, pDEST18, pDEST19, pDEST20, pDEST21, pDEST22, pDEST23, pDEST24, pDEST25, pDEST26, pDEST27, pDEST28, pDEST29, pDEST30, pDEST31, pDEST32, pDEST33, pDEST34, pDONR201 (also known as pENTR21 attP vector or pAttPkan Donor Vector), pDONR202, pDONR203 (also known as pEZ15812), pDONR204, pDONR205, pDONR206 (also known as pENTR22 attP vector or pAttPgen Donor Vector), pDONR207, pMAB58, pMAB62, pMAB85 and pMAB86.

34. A host cell comprising the vector of claim 33.

35. A polypeptide encoded by the vector of claim 33.

36. A kit for use in synthesizing a nucleic acid molecule, said kit comprising the isolated nucleic acid molecule of any one of claims 1-10, 26 and 29.

37. A kit for use in synthesizing a nucleic acid molecule, said kit comprising the primer of claim 15 or claim 18.

38. A kit for use in cloning a nucleic acid molecule, said kit comprising the vector of claim 19 or claim 33.

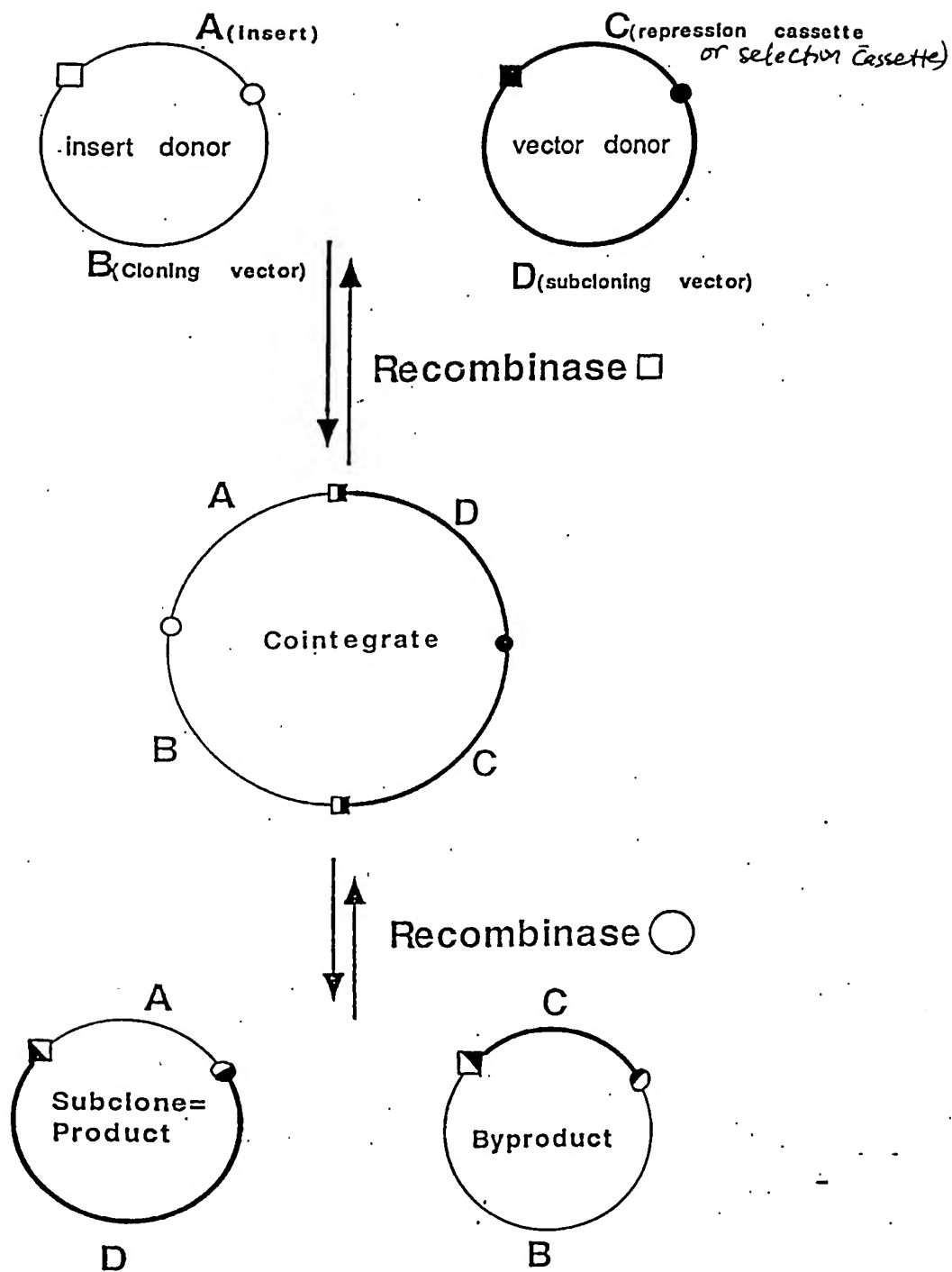


Figure 1

2/240

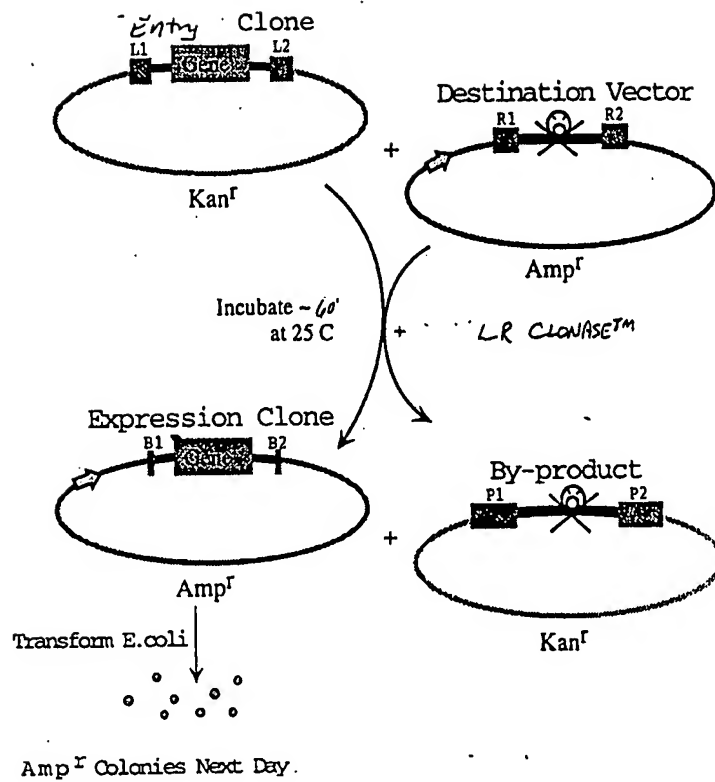


FIGURE 2



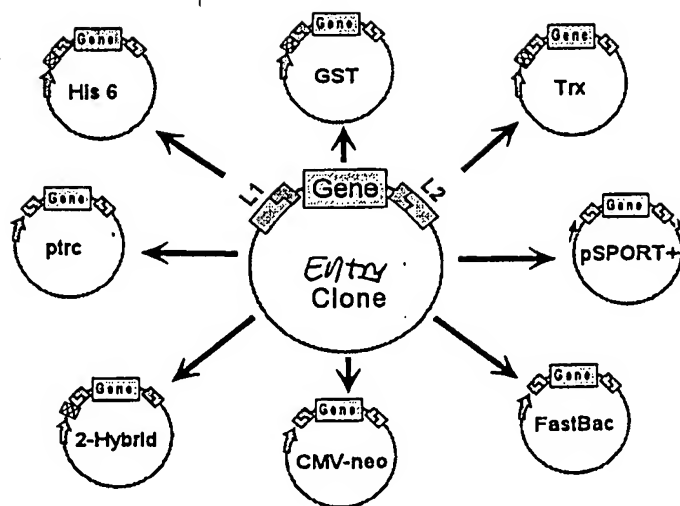


FIGURE 3

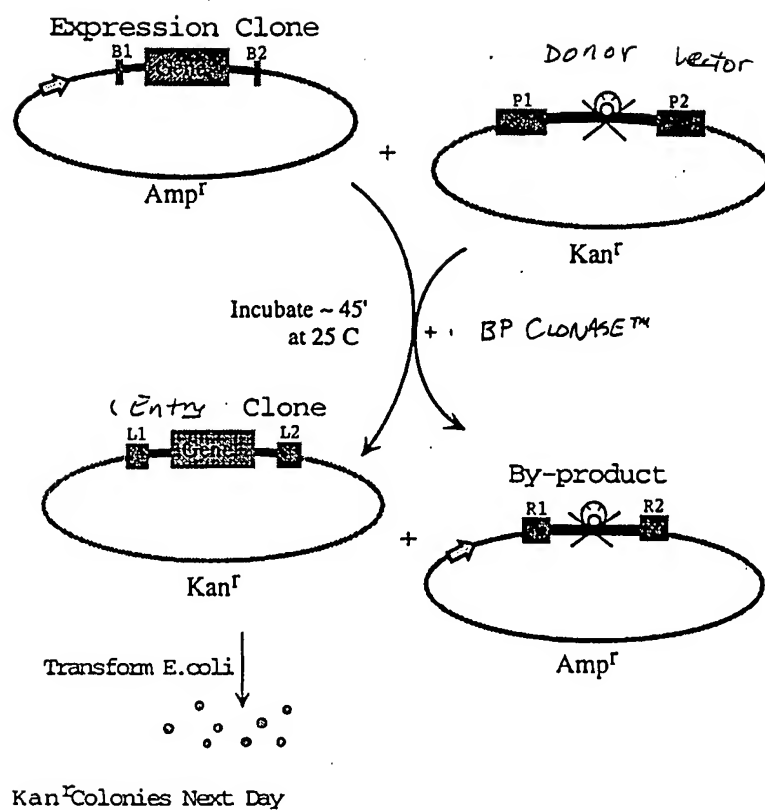


FIGURE 4

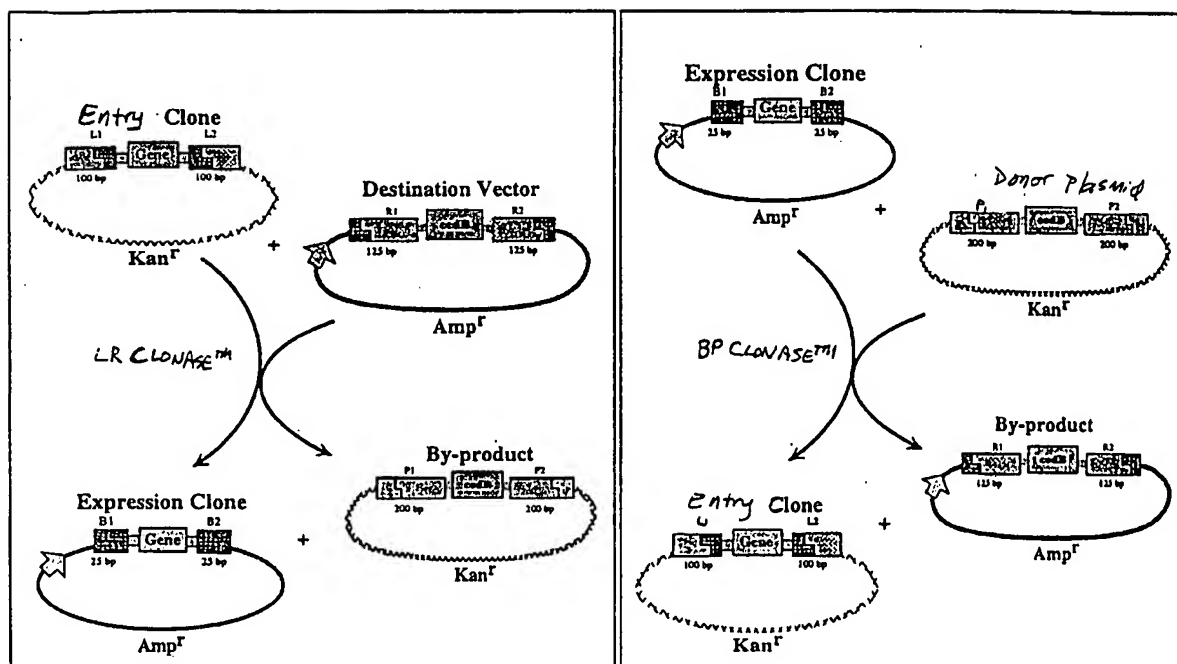


FIGURE 5

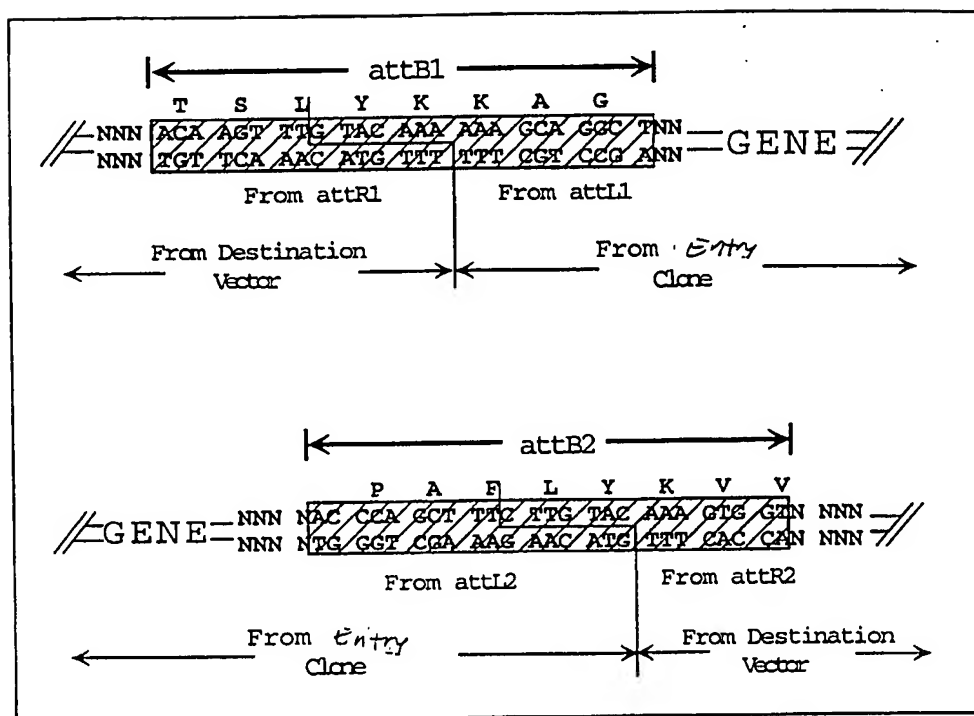


FIGURE 6

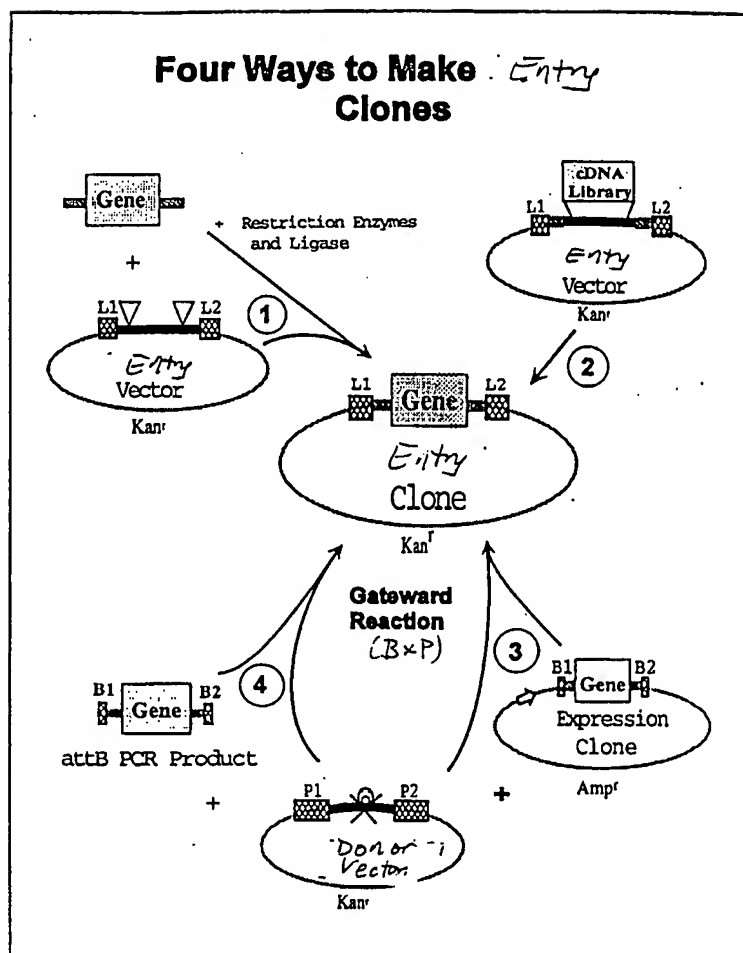


FIGURE 7

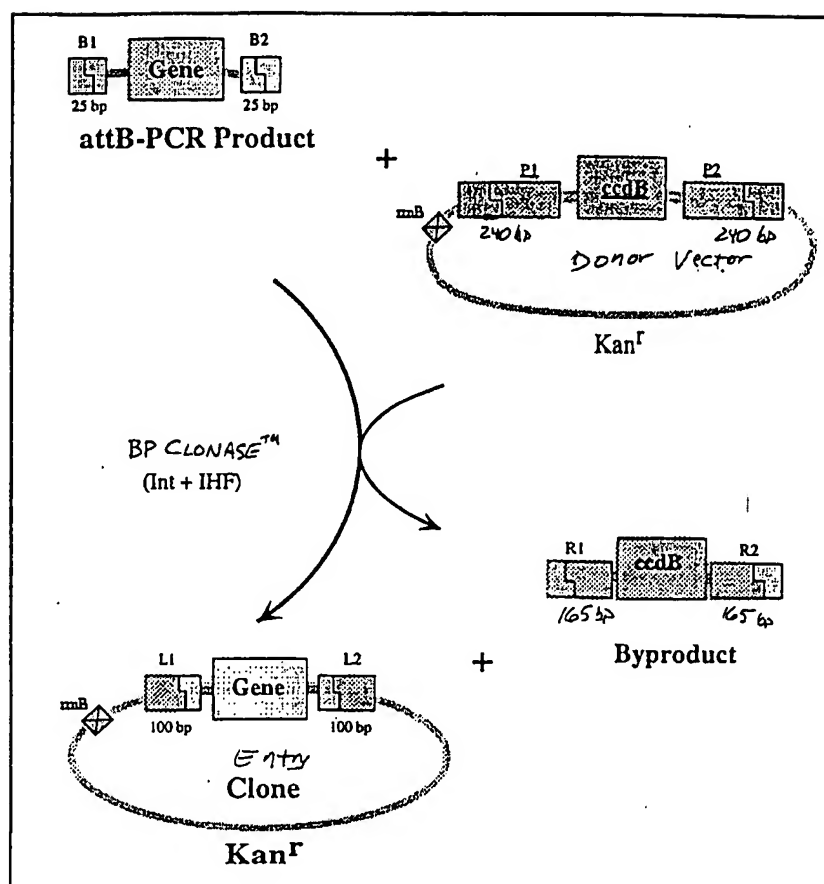


FIGURE 8

### Recombination Site Nucleotide Sequences

attB1: 5'-ACAAGTTTGTACAAAAAAGCAGGCT-3'

attB2: 5'-ACCCAGCTTTCTTGTACAAAGTGGT-3'

attP1: 5'-TACAGGTCCTAATAACCATCTAAGTAGTTGATTCATAGTGACTGGATATG-TTGTGTTTTACAGTATTATGTAGTCTGTTTTTTATGCAAAATCTAATTTA-ATATATTGATATTTATATCATTTCGTTTCTCGTTCAGCTTTTTTTGTAC-AAAGTTGGCATTATAAAAAAGCATTGCTCATCAATTTGTTGCAACGAACA-GGTCATATCAGTCAAATAAAATCATTATTTG-3'

attP2: 5'-CAAATAATGATTTTATTTTGACTGATAGTGACCTGTTTCGTTGCAACAAAT-TGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGCTGAAC-GAGAAACGTAAAATGATATAAATATCAATATATTAAATTAGATTTTGCAT-AAAAAACAGACTACATAATACTGTAAACACAACATATCCAGTCACTATGA-ATCAACTACTTAGATGGTATTAGTGACCTGTA-3'

attR1: 5'-ACAAGTTTGTACAAAAAAGCTGAACGAGAAACGTAAAATGATATAAA-TATCAATATATTAAATTAGATTTTGCATAAAAAACAGACTACATAATAC-TGTA AACACAACATATCCAGTCACTATG-3'

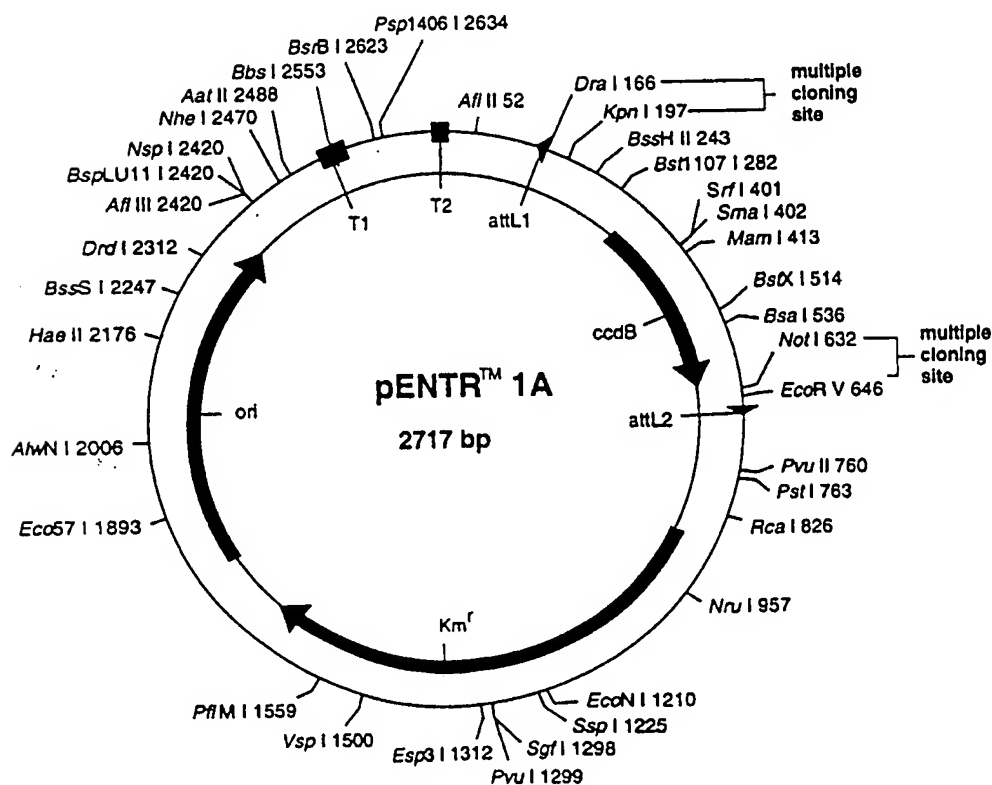
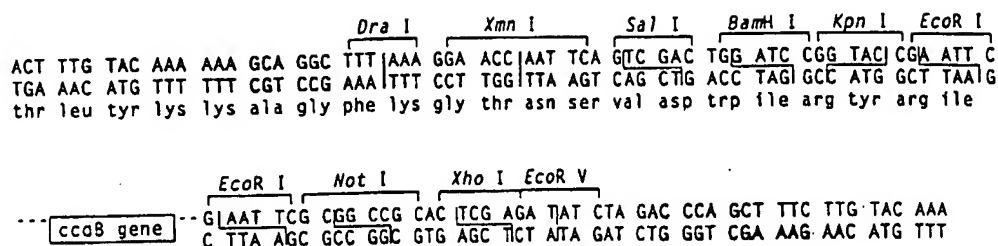
attR2: 5'-GCAGGTCGACCATAGTGACTGGATATGTTGTGTTTTACAGTATTAT-GTAGTCTGTTTTTTATGCAAAATCTAATTTAATATATTGATATTT-ATATCATTTTACGTTTCTCGTTCAGCTTTCTTGTACAAAGTGGT-3'

attL1: 5'-CAAATAATGATTTTATTTTGACTGATAGTGACCTGTTTCGTTGCAAC-AAATTGATAAGCAATGCTTTTTTATAATGCCAACTTTGTACAAAAAA-GCAGGCT-3'

attL2: 5'-CAAATAATGATTTTATTTTGACTGATAGTGACCTGTTTCGTTGCAACAA-ATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGCTGGGT-3'

Figure 9

**Figure 10A: Cloning sites of the Entry Vector pENTR1A (reading frame A)**





11/240

## pENTR1A 2717 bp

<u>Base Nos.</u>	<u>Gene Encoded</u>
67..166	attL1
321..626	ccdB
655..754	attL2
877..1686	KmR
1791..2364	ori

```

1 CTGACGGATG GCCTTTTTCG GTTCTACAA ACTCTTCCTG TTAGTTAGTT ACTTAAGCTC
61 GGGCCCCAAA TAATGATTTT ATTTTGACTG ATAGTGACCT GTTCGTTGCA ACAAATTGAT
121 AAGCAATGCT TTTTATAAAT GCCAACTTGT TACAAAAAAG CAGGCTTTAA AGGAACCAAT
181 TCAGTCGACT GGATCCGGTA CCGAATTCGC TTACTAAAAG CCAGATAACA GTATGCGTAT
241 TTGCGCGCTG ATTTTTCGGG TATAAGAATA TATACTGATA TGTATACCCG AAGTATGTCA
301 AAAAGAGGTG TGCTTCTAGA ATGCAGTTTA AGGTTTACAC CTATAAAAGA GAGAGCCGTT
361 ATCGTCTGTT TGTGGATGTA CAGAGTGATA TTATTGACAC GCCCGGGCGA CGGATAGTGA
421 TCCCCCTGGC CAGTGCACGT CTGCTGTCTG ATAAAGTCTC CCGTGAACCT TACCCGGTGG
481 TGCATATCGG GGATGAAAGC TGGCGCATGA TGACCACCGA TATGGCCAGT GTGCCGCTCT
541 CCGTTATCGG GGAAGAAGTG GCTGATCTCA GCCACCGCGA AAATGACATC AAAAACGCCA
601 TTAACCTGAT GTTCTGGGGA ATATAGAATT CGCGGCCGCA CTCGAGATAT CTAGACCCAG
661 CTTTCTTGTA CAAAGTTGGC ATTATAAGAA AGCATTGCTT ATCAATTTGT TGCAACGAAC
721 AGGTCACTAT CAGTCAAAAT AAAATCATT TTTGCCATCC AGCTGCAGCT CTGGCCCCGTG
781 TCTCAAAATC TCTGATGTTA CATTGCACAA GATAAAAATA TATCATCATG AACAATAAAA
841 CTGTCTGCTT ACATAAACAG TAATACAAGG GGTGTTATGA GCCATATTCA ACGGGAAACG
901 TCGAGGCCGC GATTAAATTC CAACATGGAT GCTGATTTAT ATGGGTATAA ATGGGCTCGC
961 GATAATGTCG GGCAATCAGG TGCGACAATC TATCGCTTGT ATGGGAAGCC CGATGCGCCA
1021 GAGTGTGTTT TGAAACATGG CAAAGGTAGC GTTGCCAATG ATGTTACAGA TGAGATGGTC
1081 AGACTAAACT GGCTGACGGA ATTTATGCCT CTTCCGACCA TCAAGCATT TATCCGTACT
1141 CCTGATGATG CATGGTTACT CACCACTGCG ATCCCCGGAA AAACAGCATT CCAGGTATTA
1201 GAAGAATATC CTGATTCAGG TGAAAATATT GTTGATGCGC TGGCAGTGTC CCTGCGCCGG
1261 TTGCATTGCA TTCCTGTTTG TAATTGTCCCT TTTAACAGCG ATCGCGTATT TCGTCTCGCT
1321 CAGGCGCAAT CACGAATGAA TAACGGTTTG GTTGATGCGA GTGATTTTGA TGACGAGCGT
1381 AATGGCTGGC CTGTTGAACA AGTCTGGAAG GAAATGCATA AACTTTTGCC ATTCTCACCG
1441 GATTCACTCG TCACTCATGG TGATTTCTCA CTTGATAACC TTATTTTGA CGAGGGGAAA
1501 TTAATAGGTT GTATTGATGT TGGACGAGTC GGAATCGCAG ACCGATACCA GGATCTTGCC
1561 ATCCTATGGA ACTGCCTCGG TGAGTTTCTT CCTTCATTAC AGAAACGGCT TTTTCAAAAA
1621 TATGGTATTG ATAATCCTGA TATGAATAAA TTGCAGTTTC ATTTGATGCT CGATGAGTTT
1681 TTCTAATCAG AATTGGTTAA TTGGTTGTAA CATTATTGAG ATTGGGCCCC GTTCCACTGA
1741 GCGTCAGACC CCGTAGAAAA GATCAAAGGA TCTTCTTGAG ATCCTTTTTT TCTGCGCGTA
1801 ATCTGCTGCT TGCAAAACAA AAAACCACCG CTACCAGCGG TGGTTTGTTT GCCGGATCAA
1861 GAGCTACCAA CTCTTTTTCG GAAGGTAAGT GGCTTCAGCA GAGCGCAGAT ACCAAATACT
1921 GTTCTTCTAG TGTAGCCGTA GTTAGGCCAC CACTTCAAGA ACTCTGTAGC ACCGCCTACA
1981 TACCTCGCTC TGCTAATCCT GTTACCAGTG GCTGCTGCCA GTGGCGATAA GTCGTGTCTT
2041 ACCGGGTTGG ACTCAAGACG ATAGTTACCG GATAAGGCGC AGCGGTGCGG CTGAACGGGG
2101 GGTTCTGTGA CACAGCCCAG CTTGGAGCGA ACGACCTACA CCGAACTGAG ATACCTACAG
2161 CGTGAGCTAT GAGAAAGCGC CACGCTTCCC GAAGGGAGAA AGGCGGACAG GTATCCGGTA
2221 AGCGGCAGGG TCGGAACAGG AGAGCGCAGC AGGGAGCTTC CAGGGGGAAA CGCCTGGTAT
2281 CTTTATAGTC CTGTCGGGTT TCGCCACCTC TGAATTGAGC GTCGATTTTT GTGATGCTCG
2341 TCAGGGGGGC GGAGCCTATG GAAAAACGCC AGCAACGCGG CCTTTTACG GTTCTTGCC
2401 TTTTGCTGGC CTTTGTCTCA CATGTTCTTT CCGCGTTAT CCCCTGATTG TGTGGATAAC
2461 CGTATTACCG CTAGCATGGA TCTCGGGGAC GTCTAACTAC TAAGCGAGAG TAGGGAACGTG
2521 CCAGGCATCA AATAAACGA AAGGCTCAGT CGGAAGACTG GGCCTTTCGT TTTATCTGTT
2581 GTTTGTGCGT GAACGCTCTC CTGAGTAGGA CAAATCCGCC GGGAGCGGAT TTGAACGTTG
2641 TGAAGCAACG GCCCGGAGGG TGGCGGGCAG GACGCCCCGC ATAAACTGCC AGGCATCAAA
2701 CTAAGCAGAA GGCCATC

```

FIGURE 10B

**Figure 11A: Cloning Sites of the Entry Vector pENTR2B (reading frame B)**

Int	attL1	EheI	XmnI	SalI	BamHI
TTG	TAC AAA AAA GCA GGC	TGG CGC CGG AAC	CAA TTC AGT CGA	CTG GAT CCG	
AAC	ATG TTT TTT CGT CCG	ACC GCG GCC TTG	GTT AAG TCA GCT	GAC CTA GGC	
Leu	Tyr Lys Lys Ala Gly Trp	Arg Arg Asn Gln Phe Ser Arg	Leu Asp Pro		

KpnI	EcoRI	EcoRI	NotI	XhoI	EcoRV	XbaI
GTA GCG AAT TC	ccdB	--G AAT TCG CCG CCG	CAC TCG AGA TAT	CTA GAC CCA		
CAT GGC TTA AG		C TTA AGC GCC GGC	GTG AGC TCT ATA	GAT CTG GGT		
Val Pro Asn		Asn Ser Arg Pro His Ser Arg Tyr Leu Asp Pro				

Int	attL2
GCT TTC TTG TAC AAA G	
CGA AAG AAC ATG TTT C	
Ala Phe Leu Tyr Lys	

## pENTR2B 2718 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
67..166		attL1
322..627		ccdB
656..755		attL2
878..1687		KmR
1792..2365		ori
1	CTGACGGATG GCCTTTTTCG GTTCTACAA ACTCTTCCTG TTAGTTAGTT ACTTAAGCTC	
61	GGGCCCCAAA TAATGATTTT ATTTTGACTG ATAGTGACCT GTTCGTTGCA ACAAATTGAT	
121	AAGCAATGCT TTTTATAAAT GCCAACTTTG TACAAAAAAG CAGGCTGGCG CCGGAACCAA	
181	TTCAGTCGAC TGGATCCGGT ACCGAATTCG CTTACTAAAA GCCAGATAAC AGTATGCGTA	
241	TTTGC GCGCT GATTTTTCG GTATAAGAAT ATATACTGAT ATGTATACCC GAAGTATGTC	
301	AAAAAGAGGT GTGCTTCTAG AATGCAGTTT AAGGTTTACA CCTATAAAAG AGAGAGCCGT	
361	TATCGTCTGT TTGTGGATGT ACAGAGTGAT ATTATTGACA CGCCCCGGCG ACGGATGGTG	
421	ATCCCCCTGG CCAGTGCACG TCTGCTGTCA GATAAAGTCT CCCGTGAAGT TTACCCGGTG	
481	GTGCATATCG GGGATGAAAG CTGGCGCATG ATGACCACCG ATATGGCCAG TGTGCCGGTC	
541	TCCGTTATCG GGGAGAAGT GGCTGATCTC AGCCACCGCG AAAATGACAT CAAAAACGCC	
601	ATTAACCTGA TGTCTGGGG AATATAGAAT TCGCGGCCGC ACTCGAGATA TCTAGACCCA	
661	GCTTCTTGT ACAAAGTTGG CATTATAAGA AAGCATTGCT TATCAATTTG TTGCAACGAA	
721	CAGGTCACCTA TCAGTCAAAA TAAATCATT ATTTGCCATC CAGCTGCAGC TCTGGCCCGT	
781	GTCTCAAAAT CTCTGATGTT ACATTGCACA AGATAAAAAT ATATCATCAT GAACAATAAA	
841	ACTGTCTGCT TACATAAACA GTAATACAAG GGGTGTATG AGCCATATTC AACGGGAAC	
901	GTGAGGCCG CGATTAAATT CCAACATGGA TGCTGATTTA TATGGGTATA AATGGGCTCG	
961	CGATAATGTC GGGCAATCAG GTGCGACAAT CTATCGCTTG TATGGGAAGC CCGATGCGCC	
1021	AGAGTTGTTT CTGAAACATG GCAAAGGTAG CGTTGCCAAT GATGTTACAG ATGAGATGGT	
1081	CAGACTAAAC TGGCTGACGG AATTTATGCC TCTCCGACC ATCAAGCATT TTATCCGTAC	
1141	TCCTGATGAT GCATGGTTAC TCACCACTGC GATCCCCGGA AAAACAGCAT TCCAGGTATT	
1201	AGAAGAATAT CCTGATTCAG GTGAAAATAT TGTGATGCG CTGGCAGTGT TCCTGCGCCG	
1261	GTTGCATTG ATTCTGTGTT GTAATTGTCC TTTTAACAGC GATCGCGTAT TTCGTCTCGC	
1321	TCAGGCGCAA TCACGAATGA ATAACGTTT GGTGATGCG AGTGATTTTG ATGACGAGCG	
1381	TAATGGCTGG CCTGTGAAC AAGTCTGGAA AGAAATGCAT AAACCTTTTG CATTCTCACC	
1441	GGATTCAAGT GTCACATCAT GTGATTTCTC ACTTGATAAC CTTATTTTGT ACGAGGGGAA	
1501	ATTAATAGGT TGTATTGATG TTGGACGAGT CGGAATCGCA GACCGATACC AGGATCTTGC	
1561	CATCCTATGG AACTGCCTCG GTGAGTTTTC TCCTTCATTA CAGAAACGGC TTTTCAAAA	
1621	ATATGGTATT GATAATCCTG ATATGAATAA ATTGCAGTTT CATTTGATGC TCGATGAGTT	
1681	TTTCTAATCA GAATTGGTTA ATTGGTTGTA ACATTATTCA GATTGGGCC CGTTCCTACTG	
1741	AGCGTCAGAC CCCGTAGAAA AGATCAAAGG ATCTTCTTGA GATCCTTTT TTCTGCGCGT	
1801	AATCTGCTGC TTGCAAACAA AAAAACCACC GCTACCAGCG GTGGTTTGT TTGCGGATCA	
1861	AGAGCTACCA ACTCTTTTC CGAAGGTAACT TGGCTTCAGC AGAGCGCAGA TACCAAATAC	
1921	TGTTCTTCTA GTGTAGCCGT AGTTAGGCCA CCACTTCAAG AACTCTGTAG CACCGCCTAC	
1981	ATACCTCGCT CTGCTAATCC TGTTACCACT GGCTGCTGCC AGTGGCGATA AGTCGTGTCT	
2041	TACCGGGTTG GACTCAAGAC GATAGTTACC GGATAAGGCG CAGCGTCCGG GCTGAACGGG	
2101	GGGTTCTGTC ACACAGCCCA GCTTGGAGCG AACGACCTAC ACCGAACCTGA GATACCTACA	
2161	GCGTGAGCTA TGAGAAAGCG CCACGCTTCC CGAAGGGAGA AAGGCGGACA GGTATCCGGT	
2221	AAGCGGCAGG GTCGGAACAG GAGAGCGCAC GAGGGAGCTT CCAGGGGGAA ACGCCTGGTA	
2281	TCTTTATAGT CCTGTCCGGT TTCGCCACCT CTGACTTGAG CGTCGATTTT TGTGATGCTC	
2341	GTCAGGGGGG CCGAGCCTAT GGAAAAACGC CAGCAACGCG GCCTTTTAC GGTTCCTGGC	
2401	CTTTTGCTGG CCTTTTGCTC ACATGTTCTT TCCTGCGTTA TCCCCTGATT CTGTGGATAA	
2461	CCGTATTACC GCTAGCATGG ATCTCGGGGA CGTCTAACTA CTAAGCGAGA GTAGGGAAC	
2521	GCCAGGCATC AAATAAACG AAAGGCTCAG TCGGAAGACT GGGCCTTTCG TTTTATCTGT	
2581	TGTTTGTCGG TGAACGCTCT CCTGAGTAGG ACAAATCCGC CGGGAGCGGA TTTGAACGTT	
2641	GTGAAGCAAC GGCCCGGAGG GTGGCGGGCA GGACGCCCGC CATAAACTGC CAGGCATCAA	
2701	ACTAAGCAGA AGGCCATC	

FIGURE 11B

**Figure 12A: Cloning Sites of the Entry Vector pENTR3C (reading frame C) ...**

Int	attL1		DraI		XmnI		SalI		BamHI								
TTG	TAC	AAA	AAA	GCA	GGC	TCT	TTA	AAG	GAA	CCA	ATT	CAG	TCG	ACT	GGA	TCC	GGT
AAC	ATG	TTT	TTT	CGT	CCG	AGA	ANT	TTC	CTT	GGT	TAA	GTC	AGC	TGA	CCT	AGG	CCA
Leu	Tyr	Lys	Lys	Ala	Gly	Ser	Leu	Lys	Glu	Pro	Ile	Gln	Ser	Thr	Gly	Ser	Gly

KpnI	EcoRI		PvuI		EcoRI		NotI		XhoI		EcoRV	XbaI			
ACC	GAA	TTC	GAT	CSC--	ccdB	--G	AAT	TCG	CGG	CCG	CAC	TCG	AGA	TAT	CTA
TGG	CTT	AAG	CTA	GCG		C	TTA	AGC	GCC	GGC	GTG	AGC	TCT	ATA	GAT
Thr	Glu	Phe					Asn	Ser	Arg	Pro	His	Ser	Arg	Tyr	Leu

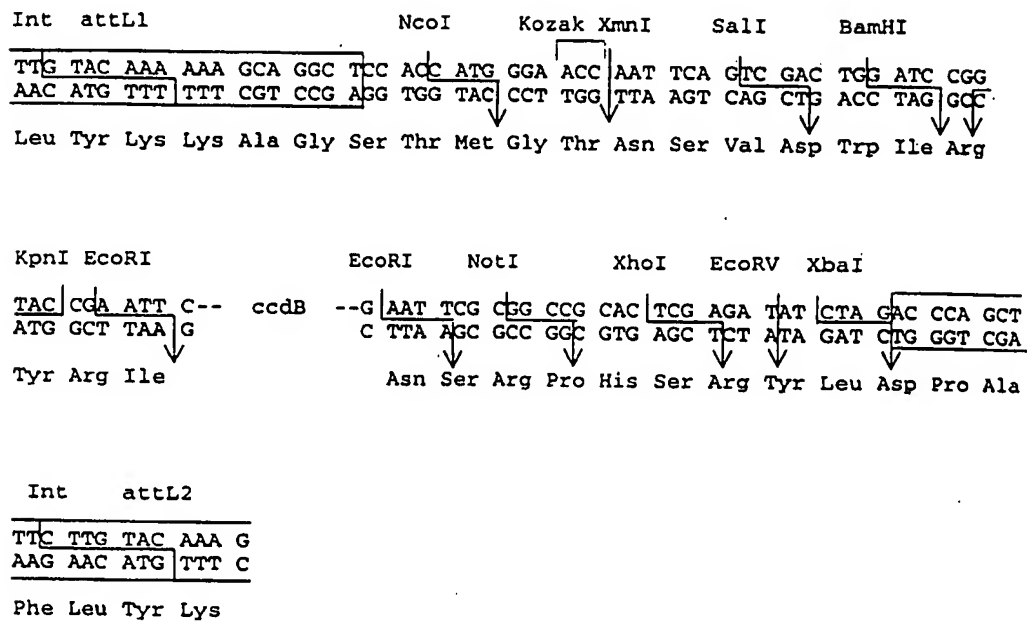
attL2	Int						
GAC	CCA	GCT	TTC	TTG	TAC	AAA	G
CTG	GGT	CGA	AAG	AAC	ATG	TTT	C
Asp	Pro	Ala	Phe	Leu	Tyr	Lys	

15/240

## pENTR3C 2723 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
67..166		attL1
327..632		ccdB
661..760		attL2
883..1692		KmR
1797..2370		ori
1	CTGACGGATG GCCTTTTTCG GTTCTACAA ACTCTTCCTG TTAGTTAGTT ACTTAAGCTC	
61	GGGCCCCAAA TAATGATTTT ATTTTGACTG ATAGTGACCT GTTCGTTGCA ACAAATTGAT	
121	AAGCAATGCT TTTTATAAT GCCAATTTC TACAAAAAAG CAGGCTCTTT AAAGGAACCA	
181	ATTCAGTCGA CTGGATCCGG TACCGAATTC GATCGCTTAC TAAAAGCCAG ATAACAGTAT	
241	GCGTATTTGC GCGCTGATTT TTGCGGTATA AGAATATATA CTGATATGTA TACCCGAAGT	
301	ATGTCAAAAA GAGGTGTGCT TCTAGAATGC AGTTTAAGGT TTACACCTAT AAAAGAGAGA	
361	GCCGTTATCG TCTGTTTGTG GATGTACAGA GTGATATTAT TGACACGCCC GGGCGACGGA	
421	TGGTGATCCC CCTGGCCAGT GCACGTCTGC TGTACAGATAA AGTCTCCCGT GAACCTTTACC	
481	CGGTGGTGCA TATCGGGGAT GAAAGCTGGC GCATGATGAC CACCGATATG GCCAGTGTGC	
541	CGGTCTCCGT TATCGGGGAA GAAGTGGCTG ATCTCAGCCA CCGCGAAAAT GACATCAAAA	
601	ACGCCATTAA CCTGATGTTT TGGGGAATAT AGAATTCGCG GCCGCACTCG AGATATCTAG	
661	ACCCAGCTTT CTTGTACAAA GTTGGCATT TAAGAAAGCA TTGCTTATCA ATTTGTTGCA	
721	ACGAACAGGT CACTATCAGT CAAAATAAAA TCATTATTTG CCATCCAGCT GCAGCTCTGG	
781	CCCGTGTCTC AAAATCTCTG ATGTTACATT GCACAAGATA AAAATATATC ATCATGAACA	
841	ATAAACTGT CTGCTTACAT AAACAGTAAT ACAAGGGGTG TTATGAGCCA TATTCAACGG	
901	GAAACGTCGA GGCCGCGATT AAATCCAAC ATGGATGCTG ATTTATATGG GTATAAATGG	
961	GCTCGCGATA ATGTCGGGCA ATCAGGTGCG ACAATCTATC GCTTGATGGA GAAGCCCCGAT	
1021	GCGCCAGAGT TGTTCCTGAA ACATGGCAAA GGTAGCGTTG CCAATGATGT TACAGATGAG	
1081	ATGGTCAGAC TAAACTGGCT GACGGAATTT ATGCCCTCTC CGACCATCAA GCATTTTATC	
1141	CGTACTCCTG ATGATGCATG GTTACTCACC ACTGCGATCC CCGGAAAAAC AGCATTCCAG	
1201	GTATTAGAAG AATATCCTGA TTCAGGTGAA AATATTGTTG ATGCGCTGGC AGTGTTCCTG	
1261	CGCCGGTTGC ATTGATTCC TGTGTGAAT TGTCCTTTTA ACAGCGATCG CGTATTTCTG	
1321	CTCGCTCAGG CGCAATCAG AATGAATAAC GGTTCGGTTG ATGCGAGTGA TTTTGATGAC	
1381	GAGCGTAATG GCTGGCCTGT TGAACAAGTC TGGAAAGAAA TGCATAAAT TTTGCCATT	
1441	TCACCGGATT CAGTCGTAC TCATGGTGAT TTCTCACTTG ATAACCTTAT TTTTGACGAG	
1501	GGGAAATTAA TAGGTTGTAT TGATGTTGGA CGAGTCGGAA TCGCAGACCG ATACCAGGAT	
1561	CTTGCCATCC TATGGAACTG CCTCGGTGAG TTTTCTCCTT CATTACAGAA ACGGCTTTTT	
1621	CAAAAATATG GTATTGATAA TCCTGATATG AATAAATTGC AGTTTCATTT GATGCTCGAT	
1681	GAGTTTCTT AATCAGAAAT GGTAAATTGG TTGTAACATT ATTCAGATTG GGCCCGGTT	
1741	CACTGAGCGT CAGACCCCGT AGAAAAGATC AAAGGATCTT CTTGAGATCC TTTTCTCTG	
1801	CGCGTAATCT GCTGCTTGA AACAAAAA CCACCGCTAC CAGCGGTGGT TTGTTTGCCG	
1861	GATCAAGAGC TACCAACTCT TTTTCCGAAG GTAACCTGGT TCAGCAGAGC GCAGATACCA	
1921	AATACTGTTT TTCTAGTGTA GCCGTAGTTA GGCCACCACT TCAAGAACTC TGTAGCACCG	
1981	CCTACATACC TCGCTCTGCT AATCCTGTTA CCAGTGGCTG CTGCCAGTGG CGATAAGTCG	
2041	TGTCTTACCG GGTTGGACTC AAGACGATAG TTACCGGATA AGGCGCAGCG GTCGGGCTGA	
2101	ACGGGGGGTT CGTGACACA GCCCAGCTTG GAGCGAACGA CCTACACCGA ACTGAGATAC	
2161	CTACAGCGTG AGCTATGAGA AAGCGCCACG CTTCCCGAAG GGAGAAAGGC GGACAGGTAT	
2221	CCGTAAGCG GCAGGGTCGG AACAGGAGAG CGCAGGAGGG AGCTTCAGG GGGAAACGCC	
2281	TGGTATCTTT ATAGTCCTGT CGGGTTTCGC CACCTCTGAC TTGAGCGTCT ATTTTGTGA	
2341	TGCTCGTCAG GGGGGCGGAG CCTATGGAAA AACGCCAGCA ACGCGGCTT TTTACGGTTC	
2401	CTGGCCTTTT GCTGGCCTTT TGCTCACATG TTCTTTCTCTG CGTTATCCCC TGATTCTGTG	
2461	GATAACCGTA TTACCGCTAG CATGGATCTC GGGGACGTCT AACTACTAAG CGAGAGTAGG	
2521	GAAGTCCAG GCATCAATA AAACGAAAGG CTCAGTCGGA AGACTGGGCC TTTCTGTTTA	
2581	TCTGTTGTTT GTCGGTGAAC GCTCTCCTGA GTAGGACAAA TCCGCCGGGA GCGGATTGTA	
2641	ACGTTGTGAA GCAACGGCCC GGAGGGTGGC GGGCAGGACG CCCGCCATAA ACTGCCAGGC	
2701	ATCAAACTAA GCAGAAGGCC ATC	

FIGURE 12B

**Figure 13A: Cloning Sites of the Entry Vector pENTR4 :**

17/240

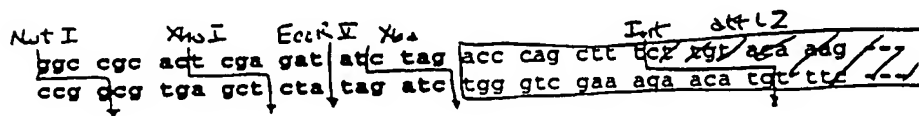
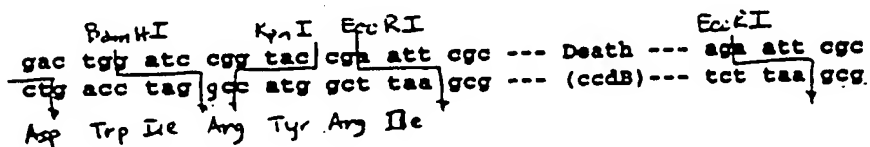
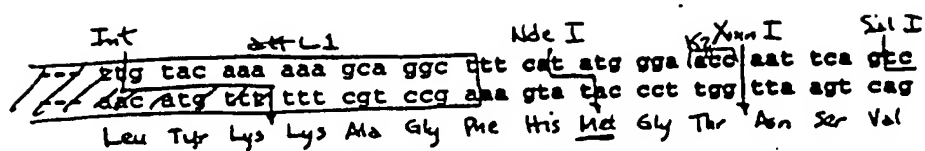
## pENTR4 2720 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
67..166		attL1
324..629		ccdB
658..757		attL2
880..1689		KmR
1794..2367		ori
1	CTGACGGATG GCCTTTTTCG GTTCTACAA ACTCTTCCTG TTAGTTAGTT ACTTAAGCTC	
61	GGGCCCCAAA TAATGATTTT ATTTTGACTG ATAGTGACCT GTTCGTTGCA ACAAATTGAT	
121	AAGCAATGCT TTTTATAAT GCCAACTTTG TACAAAAAAG CAGGCTCCAC CATGGGAACC	
181	AATTCAGTCG ACTGGATCCG GTACCGAATT CGCTTACTAA AAGCCAGATA ACAGTATGCG	
241	TATTTGCGCG CTGATTTTTCG CGGTATAAGA ATATATACTG ATATGTATAC CCGAAGTATG	
301	TCAAAAAGAG GTGTGCTTCT AGAATGCAGT TTAAGGTTTA CACCTATAAA AGAGAGAGCC	
361	GTTATCGTCT GTTTGTGGAT GTACAGAGTG ATATTATTGA CACGCCCGGG CGACGGATGG	
421	TGATCCCCCT GGCCAGTGCA CGTCTGCTGT CAGATAAAGT CTCCCGTGAA CTTTACCCGG	
481	TGGTGCATAT CGGGGATGAA AGCTGGCGCA TGATGACCAC CGATATGGCC AGTGTGCCGG	
541	TCCTCGTTAT CGGGGAAGAA GTGGCTGATC TCAGCCACCG CGAAAATGAC ATCAAAAACG	
601	CCATTAACCT GATGTCTCGG GGAATATAGA ATTCGCGGCC GCACTCGAGA TATCTAGACC	
661	CAGCTTTCTT GTACAAAGTT GGCATTATAA GAAAGCATTG CTTATCAATT TGTGTCAACG	
721	AACAGGTCAC TATCAGTCAA AATAAAATCA TTATTTGCCA TCCAGCTGCA GCTCTGGCCC	
781	GTGTCTCAA ATCTCTGATG TTACATTGCA CAAGATAAAA ATATATCATC ATGAACAATA	
841	AACTGTCTG CTTACATAAA CAGTAATACA AGGGGTGTTA TGAGCCATAT TCAACGGGAA	
901	ACGTCGAGGC CGCGATTAAA TTCCAACATG GATGCTGATT TATATGGGTA TAAATGGGCT	
961	CGCGATAATG TCGGGCAATC AGGTGCGACA ATCTATCGCT TGTATGGGAA GCCCGATGCG	
1021	CCAGAGTTGT TTCTGAAACA TGGCAAAGGT AGCGTTGCCA ATGATGTTAC AGATGAGATG	
1081	GTCAGACTAA ACTGGCTGAC GGAATTTATG CCTTTCCGA CCATCAAGCA TTTTATCCGT	
1141	ACTCCTGGTG ATGCATGGTT ACTCACCACT GCGATCCCCG GAAAAACAGC ATTCCAGGTA	
1201	TTAGAAGAAT ATCCTGATTC AGGTGAAAAT ATTGTTGATG CGCTGGCAGT GTTCTGCGC	
1261	CGGTTGCATT CGATTCTGT TTGTAATTGT CCTTTAACA GCGATCGCGT ATTTCTGCTC	
1321	GCTCAGGCGC AATCAGCAAT GAATAACGGT TTGGTTGATG CGAGTGATTT TGATGACGAG	
1381	CGTAATGGCT GGCCTGTTGA ACAAGTCTGG AAAGAAATGC ATAACTTTT GCCATTCTCA	
1441	CCGGATTAGT TCGTCACTCA TGGTGATTTC TCACTTGATA ACCTTATTTT TGACGAGGGG	
1501	AAATTAATAG GTTGTATTGA TGTGGACGA GTCGGAATCG CAGACCGATA CAGGATCTT	
1561	GCCATCCTAT GGAAGTGCCT CGGTGAGTTT TCTCCTTCAT TACAGAAACG GCTTTTTCAA	
1621	AAATATGGTA TTGATAATCC TGATATGAAT AAATGTCAGT TTCATTGAT GCTCGATGAG	
1681	TTTTTCTAAT CAGAATTGGT TAATTGGTTG TAACATTATT CAGATTGGGC CCCGTTCCAC	
1741	TGAGCGTCAG ACCCCGTAGA AAAGATCAAA GGATCTTCTT GAGATCCTTT TTTTCTGCGC	
1801	GTAATCTGCT GCTTGCAAAC AAAAAACCA CCGTACCAG CGGTGGTTTG TTTGCCGGAT	
1861	CAAGAGCTAC CAACTCTTTT TCCGAAGGTA ACTGGCTTCA GCAGAGCGCA GATACCAAAT	
1921	ACTGTTCTTC TAGTGTAGCC GTAGTTAGGC CACCACTTCA AGAACTCTGT AGCACCGCCT	
1981	ACATACCTCG CTCTGCTAAT CCTGTTACCA GTGGCTGCTG CCAGTGGCGA TAAGTCGTGT	
2041	CTTACCGGGT TGGACTCAAG ACGATAGTTA CCGGATAAGG CGCAGCGGTC GGGCTGAACG	
2101	GGGGGTTCGT GCACACAGCC CAGCTTGGAG CGAACGACCT ACACCGAACT GAGATACCTA	
2161	CAGCGTGAGC TATGAGAAAG CGCCACGCTT CCCGAAGGGA GAAAGGCGGA CAGGTATCCG	
2221	GTAAGCGGCA GGGTCGGAAC AGGAGAGCGC ACGAGGGAGC TTCCAGGGGG AAACGCGCTG	
2281	TATCTTTATA GTCCTGTCGG GTTTCGCCAC CTCTGACTTG AGCGTCGATT TTTGTGATGC	
2341	TCGTCAGGGG GGGCGAGCCT ATGGAAAAAC GCCAGCAACG CGGCCTTTT ACGGTTCTCT	
2401	GCCTTTTGCT GGCCTTTTGC TCACATGTTT TTTCTGCGT TATCCCCTGA TTCTGTGGAT	
2461	AACCGTATTA CCGCTAGCAT GGATCTCGGG GACGTCTAAC TACTAAGCGA GAGTAGGGAA	
2521	CTGCCAGGCA TCAAATAAAA CGAAAGGCTC AGTCGGAAGA CTGGGCCTTT CGTTTATCT	
2581	GTTGTTTGTG GGTGAACGCT CTCCTGAGTA GGACAAATCC GCCGGGAGCG GATTTGAACG	
2641	TTGTGAAGCA ACGGCCCGGA GGGTGGCGGG CAGGACGCCC GCCATAAACT GCCAGGCATC	
2701	AAACTAAGCA GAAGGCCATC	

FIGURE 13B

18/240

Figure 14A: Cloning sites of the Entry Vector pENTR25





19/240

## pENTR5 2720 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
67..166		attL1
324..629		ccdB
658..757		attL2
880..1689		KmR
1794..2367		ori

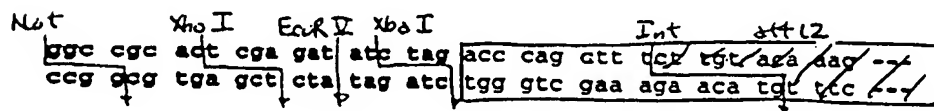
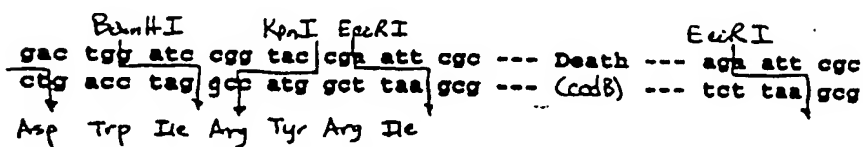
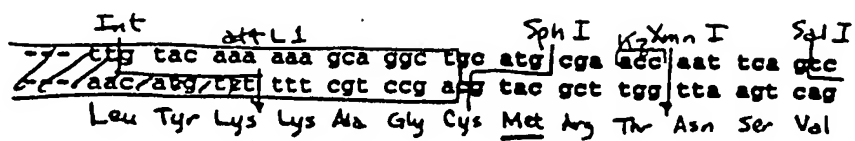
  

1	CTGACGGATG	GCCTTTTTCG	GTTTCTACAA	ACTCTTCCTG	TTAGTTAGTT	ACTTAAGCTC
61	GGGCCCCAAA	TAATGATTTT	ATTTTGACTG	ATAGTGACCT	GTTGCTTGCA	ACAAATTGAT
121	AAGCAATGCT	TTTTTATAAT	GCCAACTTTG	TACAAAAAAG	CAGGCTTTCA	TATGGGAACC
181	AATTTCAGTCG	ACTGGATCCG	GTACCGAATT	CGCTTACTAA	AAGCCAGATA	ACAGTATGCG
241	TATTTGCGCG	CTGATTTTTCG	CGGTATAAGA	ATATATACTG	ATATGTATAC	CCGAAGTATG
301	TCAAAAAGAG	GTGTGCTTCT	AGAATGCAGT	TTAAGGTTTA	CACCTATAAA	AGAGAGAGCC
361	GTTATCGTCT	GTTTGTGGAT	GTACAGAGTG	ATATTATTGA	CACGCCCGGG	CGACGGATGG
421	TGATCCCCCT	GGCCAGTGCA	CGTCTGCTGT	CAGATAAAGT	CTCCCGTGAA	CTTTACCCGG
481	TGGTGATAT	CGGGGATGAA	AGCTGGCGCA	TGATGACCAC	CGATATGGCC	AGTGTGCCGG
541	TCTCCGTTAT	CGGGGAAGAA	GTGGCTGATC	TCAGCCACCG	CGAAAATGAC	ATCAAAAACG
601	CCATTAACTT	GATGTTCTGG	GGAATATAGA	ATTGCGCGCC	GCACTCGAGA	TATCTAGACC
661	CAGCTTTCTT	GTACAAAGTT	GGCATTATAA	GAAAGCATTG	CTTATCAATT	TGTTGCAACG
721	AACAGGTCAC	TATCAGTCAA	AATAAAATCA	TTATTTGCCA	TCCAGCTGCA	GCTCTGGCCC
781	GTGTCTCAAA	ATCTCTGATG	TTACATTGCA	CAAGATAAAA	ATATATCATC	ATGAACAATA
841	AAACTGTCTG	CTTACATAAA	CAGTAATACA	AGGGGTGTTA	TGAGCCATAT	TCAACGGGAA
901	ACGTCGAGGC	CGCGATTAAA	TTCCAACATG	GATGCTGATT	TATATGGGTA	TAAATGGGCT
961	CGCGATAATG	TCGGGCAATC	AGGTGCGACA	ATCTATCGCT	TGTATGGGAA	GCCCGATGCG
1021	CCAGAGTTGT	TTCTGAAACA	TGGCAAAGGT	AGCGTTGCCA	ATGATGTTAC	AGATGAGATG
1081	GTCAGACTAA	ACTGGCTGAC	GGAATTTATG	CCTCTTCCGA	CCATCAAGCA	TTTTATCCGT
1141	ACTCCTGATG	ATGCATGGTT	ACTCACCACT	GCGATCCCCG	GAAAAACAGC	ATTCCAGGTA
1201	TTAGAAGAAT	ATCCTGATTG	AGGTGAAAAT	ATTGTTGATG	CGCTGGCAGT	GTTCTGCGCG
1261	CGGTTGCATT	CGATTCTCTG	TTGTAATTGT	CCTTTTAACA	GCGATCGCGT	ATTTCTGCTC
1321	GCTCAGGCGC	AATCACGAAT	GAATAACGGT	TTGGTTGATG	CGAGTGATTT	TGATGACGAG
1381	CGTAATGGCT	GGCCTGTTGA	ACAAGTCTGG	AAAGAAATGC	ATAAACTTTT	GCCATTCTCA
1441	CCGGATTGAG	TCGTCACTCA	TGGTGATTTC	TCATTGATA	ACCTTATTTT	TGACGAGGGG
1501	AAATTAATAG	GTTGTATTGA	TGTTGGACGA	GTCCGAATCG	CAGACCGATA	CCAGGATCTT
1561	GGCATCCTAT	GGAATCGCCT	CGGTGAGTTT	TCTCCTTCAT	TACAGAAACG	GCTTTTTCAA
1621	AAATATGGTA	TTGATAATCC	TGATATGAAT	AAATTGCAGT	TTCATTGATG	GCTCGATGAG
1681	TTTTTCTAAT	CAGAATTGGT	TAATTGGTTG	TAACATTATT	CAGATTGGGC	CCCGTTCCAC
1741	TGAGCGTCAG	ACCCCGTAGA	AAAGATCAAA	GGATCTTCTT	GAGATCCTTT	TTTTCTGCGC
1801	GTAATCTGCT	GCTTGCAAAC	AAAAAAACCA	CCGCTACCAG	CGGTGGTTTG	TTTGCCGGAT
1861	CAAGAGCTAC	CAACTCTTTT	TCCGAAGGTA	ACTGGCTTCA	GCAGAGCGCA	GATACCAAT
1921	ACTGTTCTTC	TAGTGTAGCC	GTAAGTTAGG	CACCACTTCA	AGAACTCTGT	AGCACCCCTT
1981	ACATACCTCG	CTCTGCTAAT	CCTGTTACCA	GTGGCTGCTG	CCAGTGCGCA	TAAGTCGTGT
2041	CTTACCGGGT	TGGACTCAAG	ACGATAGTTA	CCGGATAAGG	CGCAGCGGTC	GGGCTGAACG
2101	GGGGGTTGCT	GCACACAGCC	CAGCTTGGAG	CGAACGACCT	ACACCGAACT	GAGATACCTA
2161	CAGCGTGAGC	TATGAGAAAG	CGCCACGCTT	CCCGAAGGGA	GAAAGGCGGA	CAGGTATCCG
2221	GTAAGCGGCA	GGGTCCGAAC	AGGAGAGCGC	ACGAGGGAGC	TTCCAGGGGG	AAACGCCTTG
2281	TATCTTTATA	GTCCTGTCGG	GTTTCGCCAC	CTCTGACTTG	AGCGTCGATT	TTTGTGATGC
2341	TCGTACGGGG	GGCGGAGCCT	ATGGAAAAAC	GCCAGCAACG	CGGCCTTTTT	ACGGTTCTCTG
2401	GCCTTTTGCT	GGCCTTTTGC	TCACATGTTT	TTTCTGCGT	TATCCCTTGA	TTCTGTGGAT
2461	AACCGTATTA	CCGCTAGCAT	GGATCTCGGG	GACGCTTAAC	TACTAAGCGA	GAGTAGGGAA
2521	CTGCCAGGCA	TCGAATAAAA	CGAAAGGCTC	AGTCGGAAGA	CTGGGCCTTT	CGTTTTATCT
2581	TTGTGTTGTC	GGTGAACGCT	CTCCTGAGTA	GGACAAATCC	GCCGGGAGCG	GATTTGAACG
2641	TTGTGAAGCA	ACGGCCCGGA	GGGTGCGCGG	CAGGACGCCC	GCCATAAACT	GCCAGGCATC
2701	AAACTAAGCA	GAAGGCCATC				

FIGURE 14B

20/240

Figure 15A: Cloning sites of the Entry Vector pENTR6



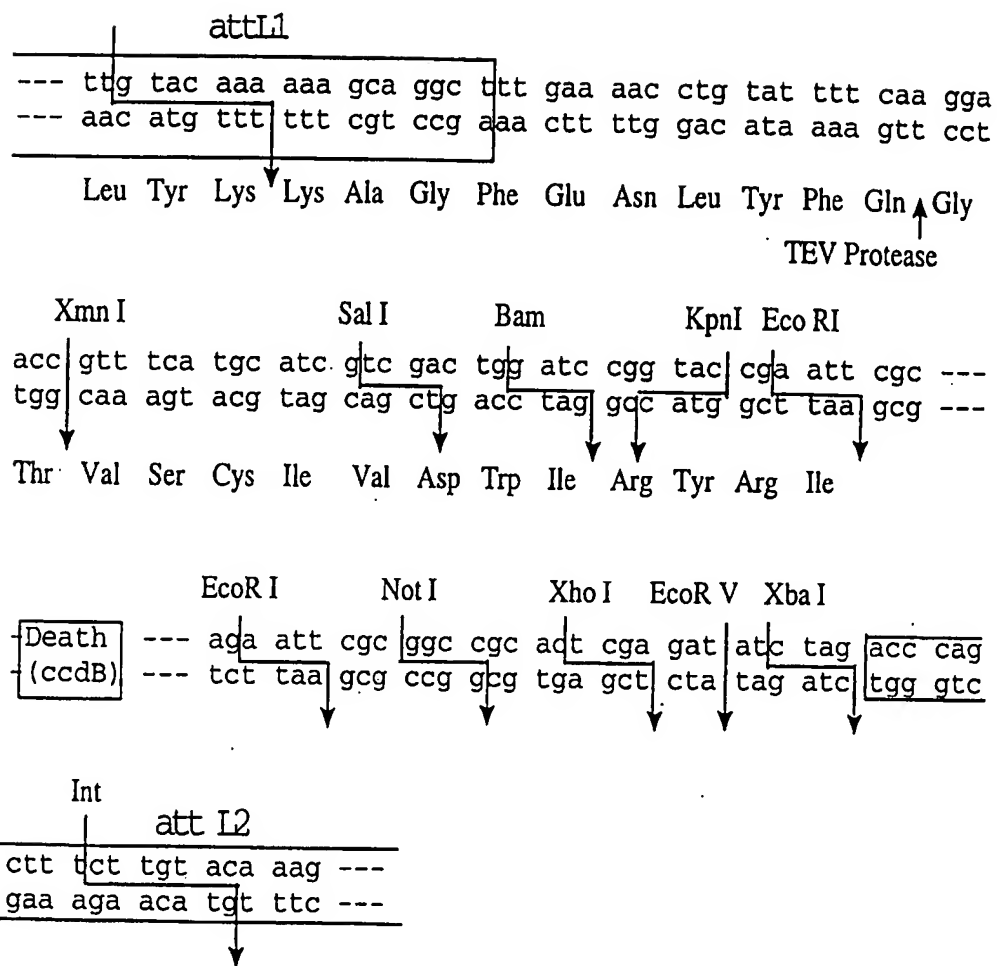
21/240

## pENTR6 2717 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
67..166		attL1
321..626		ccdB
655..754		attL2
877..1686		KmR
1791..2364		ori
1	CTGACGGATG GCCTTTTTCG GTTCTACAA ACTCTTCCTG TTAGTTAGTT ACTTAAGCTC	
61	GGGCCCCAAA TAATGATTTT ATTTTGACTG ATAGTGACCT GTTCGTTGCA ACAAATTGAT	
121	AAGCAATGCT TTTTATAAT GCCAACTTTG TACAAAAAAG CAGGCTGCAT GCGAACCAAT	
181	TCAGTCGACT GGATCCGGTA CCGAATTCGC TTACTAAAAG CCAGATAACA GTATGCGTAT	
241	TTGCGCGCTG ATTTTTCGGG TATAAGAATA TATACTGATA TGTATACCCG AAGTATGTCA	
301	AAAAGAGGTG TGCTTCTAGA ATGCAGTTTA AGGTTTACAC CTATAAAAGA GAGAGCCGTT	
361	ATCGTCTGTT TGTGGATGTA CAGAGTGATA TTATTGACAC GCCCGGGCGA CGGATGGTGA	
421	TCCCCCTGGC CAGTGCACGT CTGCTGTCAG ATAAAGTCTC CCGTGAACCT TACCCGGTGG	
481	TGCATATCGG GGATGAAAGC TGCGCGCATGA TGACCACCGA TATGGCCAGT GTGCCGGTCT	
541	CCGTTATCGG GGAAGAAGTG GCTGATCTCA GCCACCGCGA AAATGACATC AAAAACGCCA	
601	TTAACCTGAT GTTCTGGGGA ATATAGAATT CGCGGCCGCA CTCGAGATAT CTAGACCCAG	
661	CTTCTTTGTA CAAAGTTGGC ATTATAAGAA AGCATTGCTT ATCAATTTGT TGCAACGAAC	
721	AGGTCACAT CAGTCAAAAT AAAATCATT TTTGCCATCC AGCTGCAGCT CTGGCCCCGTG	
781	TCTCAAAATC TCTGATGTTA CATTGCACAA GATAAAAATA TATCATCATG AACAAATAAA	
841	CTGTCTGCTT ACATAAACAG TAATACAAGG GGTGTTATGA GCCATATTCA ACGGGAAACG	
901	TCGAGGCCGC GATTAAATTC CAACATGGAT GCTGATTTAT ATGGGTATAA ATGGGCTCGC	
961	GATAATGTCG GGCAATCAGG TGCGACAATC TATCGCTTGT ATGGGAAGCC CGATGCGCCA	
1021	GAGTTGTTTC TGAAACATGG CAAAGGTAGC GTTGCCAATG ATGTTACAGA TGAGATGGTC	
1081	AGACTAAACT GGCTGACGGA ATTTATGCCT CTCCGACCA TCAAGCATT TATCCGTACT	
1141	CCTGATGATG CATGGTTACT CACCACTGCG ATCCCCGGAA AAACAGCATT CCAGGTATTA	
1201	GAGAATATC CTGATTCAGG TGAAATATT GTTGATGCGC TGGCAGTGTT CTGCGCCGG	
1261	TTGCATTGTA TTCCTGTTTG TAATTGTCTT TTTAACAGCG ATCGCGTATT TCGTCTCGCT	
1321	CAGGCGCAAT CACGAATGAA TAACGGTTTG GTTGATGCGA GTGATTTTGA TGACGAGCGT	
1381	AATGGCTGGC CTGTTGAACA AGTCTGGAAA GAAATGCATA AACTTTTGCC ATTCTCACCG	
1441	GATTCAGTCG TCACTCATGG TGATTTCTCA CTTGATAACC TTATTTTGA CGAGGGGAAA	
1501	TTAATAGGTT GTATTGATGT TGGACGAGTC GGAATCGCAG ACCGATACCA GGATCTTGCC	
1561	ATCCTATGGA ACTGCCTCGG TGAGTTTCTT CCTTCATTAC AGAAACGGCT TTTTCAAAAA	
1621	TATGGTATTG ATAATCCTGA TATGAATAAA TTGCAGTTTC ATTTGATGCT CGATGAGTTT	
1681	TTCTAATCAG AATTGGTTAA TTGGTTGTAA CATTATTCAG ATTGGGCCCC GTTCCACTGA	
1741	GCGTCAGACC CCGTAGAAAA GATCAAAGGA TCTTCTTGAG ATCCTTTTTT TCTGCGCGTA	
1801	ATCTGCTGCT TGCAAAACAA AAAACCACCG CTACCAGCGG TGGTTTGTTC GCCGGATCAA	
1861	GAGCTACCAA CTCTTTTTC GAAGGTAACCT GGCTTCAGCA GAGCGCAGAT ACCAAATACT	
1921	GTTCTTCTAG TGTAGCCGTA GTTAGGCCAC CACTTCAAGA ACTCTGTAGC ACCGCCTACA	
1981	TACCTCGCTC TGCTAATCCT GTTACCAGTG GCTGCTGCCA GTGGCGATAA GTCGTGTCTT	
2041	ACCGGGTTGG ACTCAAGACG ATAGTTACCG GATAAGGCGC AGCGGTGCGG CTGAACGGGG	
2101	GGTTCGTGCA CACAGCCAG CTGGAGCGA ACGACCTACA CCGAACTGAG ATACCTACAG	
2161	CGTGAGCTAT GAGAAAGCGC CACGCTTCCC GAAGGGAGAA AGGCGGACAG GTATCCGGTA	
2221	AGCGGCAGGG TCGGAACAGG AGAGCGCACG AGGGAGCTTC CAGGGGGAAA CGCCTGGTAT	
2281	CTTTATAGTC CTGTGCGGTT TCGCCACCTC TGACTTGAGC GTCGATTTT GTGATGCTCG	
2341	TCAGGGGGGC GGAGCCTATG GAAAAACGCC AGCAACGCGG CCTTTTACG GTTCTGGCC	
2401	TTTTGCTGGC CTTTTGCTCA CATGTTCTTT CCTGCGTTAT CCCCTGATTC TGTGGATAAC	
2461	CGTATTACCG CTAGCATGGA TCTCGGGGAC GTCTAACTAC TAAGCGAGAG TAGGGAACCTG	
2521	CCAGGCATCA AATAAACGAA AAGGCTCAGT CGGAAGACTG GGCCTTTCGT TTTATCTGTT	
2581	GTTTGTCCGT GAACGCTCTC CTGAGTAGGA CAAATCCGCC GGGAGCGGAT TTGAACGTTG	
2641	TGAAGCAACG GCCCGGAGGG TGCGGGCAG GACGCCCCGC ATAACTGCC AGGCATCAAA	
2701	CTAAGCAGAA GGCCATC	

Figure 15B

Figure 16A: Cloning sites of the Entry Vector pENTR7



## pENTR7 2738 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
67..166		attL1
342..647		ccdB
676..775		attL2
898..1707		KmR
1812..2385		ori
1	CTGACGGATG GCCTTTTTCG GTTCTACAA ACTCTTCCTG TTAGTTAGTT ACTTAAGCTC	
61	GGGCCCCAAA TAATGATTTT ATTTTGACTG ATAGTGACCT GTTCGTTGCA ACAAATTGAT	
121	AAGCAATGCT TTTTATAAAT GCCAACTTTG TACAAAAAAG CAGGCTTTGA AAACCTGTAT	
181	TTTCAAGGAA CCGTTTCATG CATCGTCGAC TGGATCCGGT ACCGAATTCG CTTACTAAAA	
241	GCCAGATAAC AGTATGCGTA TTTGCGCGCT GATTTTTCG GTATAAGAAT ATATACTGAT	
301	ATGTATACCC GAAGTATGTC AAAAAGAGGT GTGCTTCTAG AATGCAGTTT AAGGTTTACA	
361	CCTATAAAAG AGAGAGCCGT TATCGTCTGT TTGTGGATGT ACAGAGTGAT ATTATTGACA	
421	CGCCCCGGCG ACGGATAGTG ATCCCCCTGG CCAGTGCACG TCTGCTGTCA GATAAAGTCT	
481	CCCCTGAAC TACCCCGGTG GTGCATATCG GGGATGAAAG CTGGCGCATG ATGACCACCG	
541	ATATGGCCAG TGTGCCGGTC TCCGTTATCG GGAAGAAGT GGCTGATCTC AGCCACCGCG	
601	AAAATGACAT CAAAAACGCC ATTAACCTGA TGTTCGCGG AATATAGAAT TCGCGGCCGC	
661	ACTCGAGATA TCTAGACCCA GCTTCTTGT ACAAAGTTGG CATTATAAGA AAGCATTGCT	
721	TATCAATTTG TTGCAACGAA CAGGTCAC TAAGTCAAAA TAAAATCATT ATTTGCCATC	
781	CAGCTGCAGC TCTGGCCCGT GTCTCAAAAT CTCTGATGTT ACATTGCACA AGATAAAAAAT	
841	ATATCATCAT GAACAATAAA ACTGCTGTCT TACATAAACA GTAATACAAG GGGTGTATG	
901	AGCCATATTC AACGGGAAAC GTCGAGGCCG CGATTAAATT CCAACATGGA TGCTGATTTA	
961	TATGGGTATA AATGGGCTCG CGATAATGTC GGGCAATCAG GTGCGACAAT CTATCGCTTG	
1021	TATGGGAAGC CCGATGCGCC AGAGTTGTTT CTGAAACATG GCAAAGGTAG CGTTGCCAAT	
1081	GATGTTACAG ATGAGATGGT CAGACTAAAC TGGCTGACGG AATTTATGCC TCTTCCGACC	
1141	ATCAAGCATT TTATCCGTAC TCCTGATGAT GCATGGTTAC TCACCACTGC GATCCCCGGA	
1201	AAAACAGCAT TCCAGGTATT AGAAGAATAT CCTGATTCAG GTGAAAATAT TGTGTATGCG	
1261	CTGGCAGTGT TCCTGCGCCG GTTGCAATTC ATTCTGTGTT GTAATTGTCC TTTTAACAGC	
1321	GATCGCGTAT TTCGTCTCGC TCAGGCGCAA TCACGAATGA ATAACGGTTT GGTGTATGCG	
1381	AGTGATTTTG ATGACGAGCG TAATGGCTGG CCTGTGAAC AAGTCTGGAA AGAAATGCAT	
1441	AACTTTTTCG CATTCTCACC GGATTCAGTC GTCACATCAT GTGATTCTCT ACTTGATAAC	
1501	CTTATTTTTC ACGAGGGGAA ATTAATAGGT TGTATTGATG TTGGACGAGT CGGAATCGCA	
1561	GACCGATACC AGGATCTTGC CATCCCTATG AACTGCCTCG GTGAGTTTTC TCCTTCAATTA	
1621	CAGAAACGGC TTTTTCAAAA ATATGGTATT GATAATCCTG ATATGAATAA ATTGCAGTTT	
1681	CATTTGATGC TCGATGAGTT TTTCTAATCA GAATTGGTTA ATTGGTTGTA ACATTATTCA	
1741	GATTGGGCCC CGTTCCACTG AGCGTCAGAC CCCGTAGAAA AGATCAAAGG ATCTTCTTGA	
1801	GATCCTTTTT TTCTGCGCGT AATCTGCTGC TTGCAAAACA AAAAACCACC GCTACCAGCG	
1861	GTGGTTTGTT TGCCGGATCA AGAGCTACCA ACTCTTTTTT CGAAGGTAAC TGGCTTCAGC	
1921	AGAGCGCAGA TACCAATAC TGTCTTTCTA GTGTAGCCGT AGTTAGGCCA CCACCTCAAG	
1981	AACTCTGTAG CACCGCCTAC ATACCTCGCT CTGCTAATCC TGTTACCAGT GGCTGCTGCC	
2041	AGTGGCGATA AGTCGTGTCT TACCGGGTTG GACTCAAGAC GATAGTTACC GGATAAGGCG	
2101	CAGCGGTCGG GCTGAACGGG GGGTTCGTGC ACACAGCCCA GCTTGGAGCG AACGACCTAC	
2161	ACCGAACTGA GATACCTACA GCGTGAGCTA TGAGAAAGCG CCACGCTTCC CGAAGGGAGA	
2221	AAGGCGGACA GGTATCCGGT AAGCGGCAGG GTCGGAACAG GAGAGCGCAC GAGGGAGCTT	
2281	CCAGGGGGAA ACGCCTGGTA TCTTTATAGT CCTGTCGGGT TTCGCCACCT CTGACTTGAG	
2341	CGTCGATTTT TGTGATGCTC GTCAGGGGGG CGGAGCCTAT GGAAAAACGC CAGCAACGCG	
2401	GCCTTTTAC GGTTCCTGGC CTTTGTCTGG CTTTGTCTC ACATGTTCTT TCCTGCGTTA	
2461	TCCCTTGATT CTGTGGATAA CCGTATTACC GCTAGCATGG ATCTCGGGGA CGTCTAATA	
2521	CTAAGCGAGA GTAGGGAAC TCCAGGCATC AAATAAAACG AAAGGCTCAG TCGGAAGACT	
2581	GGGCCTTTTC TTTTATCTGT TGTGTGTCGG TGAACGCTCT CCTGAGTAGG ACAAATCCGC	
2641	CGGAGCGGGA TTTGAACGTT GTGAAGCAAC GGCCCGGAGG GTGGCGGGCA GGACGCCCGC	
2701	CATAAACTGC CAGGCATCAA ACTAAGCAGA AGGCCATC	

FIGURE 16B

24/260

Figure 17A: Cloning Sites of the *ENTRY* Vector, pENTRYB

Int attC1

<del>ctt</del> <del>tac</del> <del>aaa</del> <del>aaa</del> <del>gca</del> <del>ggc</del> <del>ttt</del>	<del>gaa</del> <del>aac</del> <del>ctg</del> <del>tat</del> <del>ttt</del> <del>caa</del> <del>gaa</del>
<del>gag</del> <del>gtg</del> <del>gtc</del> <del>ttt</del> <del>cgt</del> <del>ccg</del> <del>aaa</del>	<del>ctt</del> <del>ttg</del> <del>gac</del> <del>ata</del> <del>aaa</del> <del>gtt</del> <del>cct</del>
Leu Tyr Lys Lys Ala Gly Phe Glu Asn Leu Tyr Phe Gln Gly	

TEV Protease

NotI	Ara II	SaI	BamHI	KpnI	EcoRI
<del>act</del> <del>atg</del> <del>gac</del> <del>cta</del> <del>gtc</del> <del>gac</del> <del>tgg</del> <del>atc</del> <del>cgg</del> <del>tac</del> <del>cga</del> <del>att</del> <del>cgc</del> ---					
<del>tgg</del> <del>tac</del> <del>ctg</del> <del>gat</del> <del>cag</del> <del>cgg</del> <del>aac</del> <del>tag</del> <del>gct</del> <del>atg</del> <del>gct</del> <del>taa</del> <del>gcg</del> ---					
Thr Met Asp Leu Val Asp Trp Ile Arg Tyr Arg Ile					

EcoRI	NotI	XhoI	EcoRI	XbaI	attC
Death --- <del>aga</del> <del>att</del> <del>cgc</del> <del>ggc</del> <del>cgc</del> <del>act</del> <del>cga</del> <del>gat</del> <del>atc</del> <del>tag</del> <del>acc</del> <del>cag</del>					
--- <del>tct</del> <del>taa</del> <del>gcg</del> <del>ccg</del> <del>ggc</del> <del>tga</del> <del>gct</del> <del>cta</del> <del>tag</del> <del>atc</del> <del>tgg</del> <del>gtc</del>					

Int

<del>ctt</del> <del>tct</del> <del>ggt</del> <del>aca</del> <del>aga</del> <del>---</del>
<del>gaa</del> <del>aga</del> <del>aca</del> <del>tgt</del> <del>ttc</del> <del>---</del>

25/240

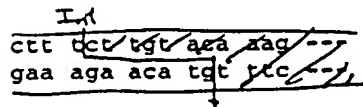
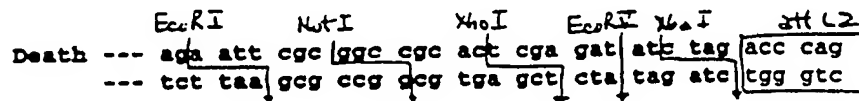
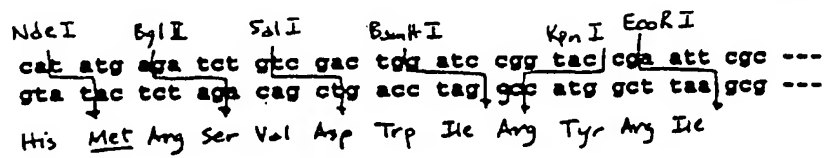
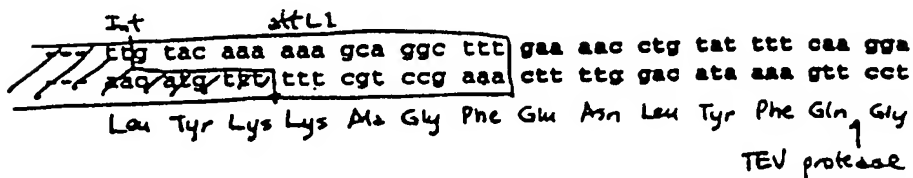
## pENTR8 2735 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
67..166		attL1
339..644		ccdB
673..772		attL2
895..1704		KmR
1809..2382		ori
1	CTGACGGATG GCCTTTTTCG GTTTCTACAA ACTCTTCCTG TTAGTTAGTT ACTTAAGCTC	
61	GGGCCCCAAA TAATGATTTT ATTTTGACTG ATAGTGACCT GTTCGTTGCA ACAAATTGAT	
121	AAGCAATGCT TTTTATAAT GCCAACTTTG TACAAAAAAG CAGGCTTTGA AAACCTGTAT	
181	TTTCAAGGAA CCATGGACCT AGTCGACTGG ATCCGGTACC GAATTCGCTT ACTAAAAGCC	
241	AGATAACAGT ATGCGTATTT GCGCGCTGAT TTTTGCGGTA TAAGAATATA TACTGATATG	
301	TATACCCGAA GTATGTCAAA AAGAGGTGTG CTTCTAGAAT GCAGTTTAAG GTTTACACCT	
361	ATAAAAGAGA GAGCCGTTAT CGTCTGTTTG TGGATGTACA GAGTGATATT ATTGACACGC	
421	CCGGGCGACG GATAGTGATC CCCCTGGCCA GTGCACGCTT GCTGTCAGAT AAAGTCTCCC	
481	GTGAACCTTA CCCGGTGGTG CATATCGGGG ATGAAAGCTG GCGCATGATG ACCACCGATA	
541	TGGCCAGTGT GCCGCTCTCC GTTATCGGGG AAGAAAGTGGC TGATCTCAGC CACCGCGAAA	
601	ATGACATCAA AAACGCCATT AACCTGATGT TCTGGGGAAT ATAGAATTCTG CGGCCGCACT	
661	CGAGATATCT AGACCCAGCT TTCTTGATACA AAGTTGGCAT TATAAGAAAG CATTGCTTAT	
721	CAATTTGTTG CAACGAACAG GTCACATCA GTCAAAATAA AATCATTATT TGCCATCCAG	
781	CTGCAGCTCT GGCCCGTGTC TCAAAATCTC TGATGTTACA TTGCACAAGA TAAAAATATA	
841	TCATCATGAA CAATAAAACT GTCTGCTTAC ATAAACAGTA ATACAAAGGG TGTTATGAGC	
901	CATATTCAAC GGGAAACGTC GAGGCCGCGA TTAAATTCCA ACATGGATGC TGATTTATAT	
961	GGGTATAAAT GGGCTCGCGA TAATGTCGGG CAATCAGGTG CGACAATCTA TCGCTTGAT	
1021	GGGAAGCCCG ATGCGCCAGA GTTGTTTCTG AAACATGGCA AAGGTAGCGT TGCCAATGAT	
1081	GTTACAGATG AGATGGTCAG ACTAACTGG CTGACGGAAT TTATGCCTCT TCCGACCATC	
1141	AAGCATTTTA TCCGTACTCC TGATGATGCA TGGTTACTCA CCACCTGCGAT CCCCAGAAAA	
1201	ACAGCATTCC AGGTATTAGA AGAATATCCT GATTCAGGTG AAAATATTGT TGATGCGCTG	
1261	GCAGTGTCCT TGCGCCGGTT GCATTGCGATT CCTGTTTGTA ATTGTCCTTT TAACAGCGAT	
1321	CGCGTATTTT GTCTCGCTCA GCGCAATCA CGAATGAATA ACGGTTTGGT TGATGCGAGT	
1381	GATTTTGATG ACGAGCGTAA TGGCTGGCCT GTTGAACAAG TCTGGAAAGA AATGCATAAA	
1441	CTTTTGCCAT TCTCACCGBA TTCAGTCGTC ACTCATGGTG ATTTCTCACT TGATAACCTT	
1501	ATTTTGTAGC AGGGGAAATT AATAGGTTGT ATTGATGTTG GACGAGTCGG AATCGCAGAC	
1561	CGATACCAGG ATCTTGCCAT CCTATGGAAC TGCCCTCGGTG AGTTTTCTCC TTCATTACAG	
1621	AAACGGCTTT TTCAAAAATA TGGTATTGAT AATCCTGATA TGAATAAATT GCAGTTTCAT	
1681	TTGATGCTCG ATGAGTTTTT CTAATCAGAA TTGGTTAATT GGTGTGAACA TTATTAGAT	
1741	TGGGCCCCGT TCCACTGAGC GTCAGACCCC GTAGAAAAGA TCAAAGGATC TTCTTGAGAT	
1801	CCTTTTTTTC TGCGCGTAAT CTGCTGCTTG CAAACAAAAA AACCACCGCT ACCAGCGGTG	
1861	GTTTGTTTGC CGGATCAAGA GCTACCAACT CTTTTTCCGA AGGTAACCTG CTTACGAGA	
1921	GCGCAGATAC CAAATACTGT TCTTCTAGTG TAGCCGTAGT TAGGCCACCA CTTCAAGAAC	
1981	TCTGTAGCAC CGCCTACATA CCTCGCTCTG CTAATCCTGT TACCAGTGGC TGCTGCCAGT	
2041	GGCGATAAGT CGTGTCTTAC CGGGTTGGAC TCAAGACGAT AGTTACCGGA TAAGGCGCAG	
2101	CGGTCGGGCT GAACGGGGG TTCGTGCACA CAGCCCAGCT TGGAGCGAAC GACCTACACC	
2161	GAACTGAGAT ACCTACAGCG TGAGCTATGA GAAAGCGCCA CGCTTCCCGA AGGGAGAAAG	
2221	GCGGACAGGT ATCCGGTAAG CGGCAGGGTC GGAACAGGAG AGCGCACGAG GGAGCTTCCA	
2281	GGGGGAAACG CCTGGTATCT TTATAGTCCT GTCGGGTTTC GCCACCTCTG ACTTGAGCGT	
2341	CGATTTTTGT GATGCTCGTC AGGGGGGCGG AGCCTATGGA AAAACGCCAG CAACGCGGCC	
2401	TTTTTACGGT TCCTGGCCTT TTGCTGGCCT TTTGCTCACA TGTTCTTTCC TGCGTTATCC	
2461	CCTGATTCTG TGGATAACCG TATTACCGCT AGCATGGATC TCGGGGACGT CTAACACTA	
2521	AGCGAGAGTA GGGAACTGCC AGGCATCAAA TAAAACGAAA GGCTCAGTCG GAAGACTGGG	
2581	CCTTTCGTTT TATCTGTTGT TTGTCGGTGA ACGCTCTCCT GAGTAGGACA AATCCGCCG	
2641	GAGCGGATTT GAACGTTGTG AAGCAACGGC CCGGAGGGTG GCGGGCAGGA CGCCGCCCAT	
2701	AAACTGCCAG GCATCAAACT AAGCAGAAGG CCATC	

FIGURE 17B

26/240

Figure 18A: Cloning sites of the ENTRY Vector pENTRY





27/240

## pENTR9 2735 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
67..166		attL1
339..644		ccdB
673..772		attL2
895..1704		KmR
1809..2382		ori
1	CTGACGGATG GCCTTTTTCG GTTTCTACAA ACTCTTCCTG TTAGTTAGTT ACTTAAGCTC	
61	GGGCCCCAAA TAATGATTTT ATTTTGACTG ATAGTGACCT GTTCGTTGCA ACAAATTGAT	
121	AAGCAATGCT TTTTATAAT GCCAACTTTG TACAAAAAAG CAGGCTTTGA AAACCTGTAT	
181	TTTCAAGGAC ATATGAGATC TGTCGACTGG ATCCGGTACC GAATTCGCTT ACTAAAAGCC	
241	AGATAACAGT ATGCGTATTT GCGCGCTGAT TTTTGCGGTA TAAGAATATA TACTGATATG	
301	TATACCCGAA GTATGTCAAA AAGAGGTGTG CTTCTAGAAT GCAGTTTAAG GTTTACACCT	
361	ATAAAAGAGA GAGCCGTTAT CGTCTGTTTG TGGATGTACA GAGTGATATT ATTGACACGC	
421	CCGGGCGACG GATAGTGATC CCCCTGGCCA GTGCACGTCT GCTGTCAGAT AAAGTCTCCC	
481	GTGAACCTTA CCCGGTGGTG CATATCGGGG ATGAAAGCTG GCGCATGATG ACCACCGATA	
541	TGGCCAGTGT GCCGGTCTCC GTTATCGGGG AAGAAGTGGC TGATCTCAGC CACCGCGAAA	
601	ATGACATCAA AAACGCCATT AACCTGATGT TCTGGGGAAT ATAGAATTCG CGGCCGCACT	
661	CGAGATATCT AGACCCAGCT TTCTTGATACA AAGTTGGCAT TATAAGAAAG CATTGCTTAT	
721	CAATTTGTTG CAACGAACAG GTCACATCA GTCAAAATAA AATCATTATT TGCCATCCAG	
781	CTGCAGCTCT GGCCCGTGTC TCAAAATCTC TGATGTTACA TTGCACAAGA TAAAAATATA	
841	TCATCATGAA CAATAAAACT GTCTGCTTAC ATAAACAGTA ATACAAGGGG TGTTATGAGC	
901	CATATTCAAC GGGAAACGTC GAGGCCGCGA TTAAATTCCA ACATGGATGC TGATTTATAT	
961	GGGTATAAAT GGGCTCGCGA TAATGTCGGG CAATCAGGTG CGACAATCTA TCGCTTGAT	
1021	GGGAAGCCCG ATGCGCCAGA GTTGTTCCTG AAACATGGCA AAGGTAGCGT TGCCAATGAT	
1081	GTTACAGATG AGATGGTCAG ACTAACTGG CTGACGGAAT TTATGCCTCT TCCGACCATC	
1141	AAGCATTTTA TCCGTACTCC TGATGATGCA TGGTTACTCA CCACTGCGAT CCCC GGAAAA	
1201	ACAGCATTCG AGGTATTAGA AGAATATCCT GATTCAGGTG AAAATATTGT TGATGCGCTG	
1261	GCAGTGTCCC TGCGCCGTT GCATTCGATT CCTGTTTGTA ATTGTCCTTT TAACAGCGAT	
1321	CGCGTATTTT GTCTCGCTCA GGC GCAATCA CGAATGAATA ACGGTTTGGT TGATGCGAGT	
1381	GATTTTGATG ACGAGCGTAA TGGCTGGCCT GTTGAACAAG TCTGGAAAGA AATGCATAAA	
1441	CTTTTGCCAT TCTCACC GGA TTCAGTCGTC ACTCATGGTG ATTTCTCACT TGATAACCTT	
1501	ATTTTGTGACG AGGGGAAATT AATAGGTTGT ATTGATGTTG GACGAGTCGG AATCGCAGAC	
1561	CGATACCAGG ATCTTGCCAT CCTATGGAAC TGCCCTCGTG AGTTTCTCTC TTCATTACAG	
1621	AAACGGCTTT TTCAAAAATA TGGTATTGAT AATCCTGATA TGAATAAATT GCAGTTTCAT	
1681	TTGATGCTCG ATGAGTTTTT CTAATCAGAA TTGGTTAATT GGTGTAACA TTATTACAGAT	
1741	TGGGCCCCGT TCCACTGAGC GTCAGACCCC GTAGAAAAGA TCAAAGGATC TTCTTGAGAT	
1801	CCTTTTTC TCGCGTAAT CTGCTGCTTG CAAACAAAAA AACCACCGCT ACCAGCGGTG	
1861	GTTTGTTCG CCGATCAAGA GCTACCAACT CTTTTCCGA AGGTAACCTG CTTACGACAGA	
1921	GCGCAGATAC CAAATACTGT TCTTCTAGTG TAGCCGTAGT TAGGCCACCA CTTCAAGAAC	
1981	TCTGTAGCAC CGCCTACATA CCTCGCTCTG CTAATCCTGT TACCAGTGGC TGCTGCCAGT	
2041	GGCGATAAGT CGTGCTTAC CGGGTTGGAC TCAAGACGAT AGTTACCGGA TAAGGCGCAG	
2101	CGGTCGGGCT GAACGGGGG TTCGTGCACA CAGCCCAGCT TGGAGCGAAC GACCTACACC	
2161	GAACTGAGAT ACCTACAGCG TGAGCTATGA GAAAGCGCCA CGCTTCCCGA AGGGAGAAAG	
2221	GCGGACAGGT ATCCGGTAAG CGGCAGGGTC GGAACAGGAG AGCGCACGAG GGAGCTTCCA	
2281	GGGGGAAACG CCTGGTATCT TTATAGTCCT GTCGGGTTTC GCCACCTCTG ACTTAGCGT	
2341	GATTTTTTGT GATGCTCGTC AGGGGGCGG AGCCTATGGA AAAACGCCAG CAACGCGGCC	
2401	TTTTTACGGT TCCTGGCCTT TTGCTGGCCT TTTGCTCACA TGTTCTTTCC TGCGTTATCC	
2461	CCTGATTCTG TGGATAACCG TATTACCGCT AGCATGGATC TCGGGGACGT CTAACTACTA	
2521	AGCGAGAGTA GGGAACTGCC AGGCATCAAA TAAAACGAAA GGCTCAGTCG GAAGACTGGG	
2581	CCTTTCGTTT TATCTGTTGT TTGTCGGTGA ACGCTCTCCT GAGTAGGACA AATCCGCCGG	
2641	GAGCGGATTT GAACGTTGTG AAGCAACGGC CCGGAGGGTG GCGGGCAGGA CGCCGCCCAT	
2701	AAACTGCCAG GCATCAAACT AAGCAGAAGG CCATC	

FIGURE 18B

28/240

Figure 19A: Cloning sites of the ENTRY Vector pENTRY10

Int                      attC1                      S.D.                      -12                      Nde

--- ~~ctg tac aaa aaa gca ggc ttc gaa cta~~ ~~agg aaa tac tta cat~~ ---  
 --- ~~aac atg ttc~~ ~~ttt cgt ccg aag ctt gat tcc ttt atg aat gta~~ ---  
 Leu Tyr Lys Lys Ala Gly Phe Glu Leu Arg Lys Tyr Leu His

K3 Xba                      Sma                      Bam                      Kpn                      EcoRI

atg gga acc aat tca gtc gac tgg atc cgg tac cga att cgc ---  
 tac cct tgg tta agt cag cgg acc tag gcp atg gct taa ggc ---  
 Met Gly Thr Asn Ser Val Asp Trp Ile Arg Tyr Arg Ile

EcoRI                      Not                      Xho                      EcoRII                      Xba                      attL2

Death --- aga att cgc ggc cgc act cga gat atc tag acc cag  
 (ccdB) --- tct taa gcg ccg gcg tga gct cta tag atc tgg gtc

Int

ctt tct tgg aca aag tga  
 gaa aga aca tgc tgc ---

29/240

## pENTR10 2738 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
67..166		attL1
342..647		ccdB
676..775		attL2
898..1707		KmR
1812..2385		ori

1	CTGACGGATG	GCCTTTTTCG	GTTTCTACAA	ACTCTTCCTG	TTAGTTAGTT	ACTTAAGCTC
61	GGGCCCCAAA	TAATGATTTT	ATTTTGACTG	ATAGTGACCT	GTTTCGTTGCA	ACAAATTGAT
121	AAGCAATGCT	TTTTTATAAT	GCCAACTTTG	TACAAAAAAG	CAGGCTTCGA	ACTAAGGAAA
181	TACTTACATA	TGGGAACCAA	TTCAGTCGAC	TGGATCCGGT	ACCGAATTCG	CTTACTAAAA
241	GCCAGATAAC	AGTATGCGTA	TTTGCGCGCT	GATTTTTGCG	GTATAAGAAT	ATATACTGAT
301	ATGTATACCC	GAAGTATGTC	AAAAAGAGGT	GTGCTTCTAG	AATGCAGTTT	AAGGTTTACA
361	CCTATAAAAG	AGAGAGCCGT	TATCGTCTGT	TTGTGGATGT	ACAGAGTGAT	ATTATTGACA
421	CGCCCGGGCG	ACGGATGGTG	ATCCCCCTGG	CCAGTGCACG	TCTGCTGTCA	GATAAAGTCT
481	CCCGTGAACT	TTACCCGGTG	GTGCATATCG	GGGATGAAAG	CTGGCGCATG	ATGACCACCG
541	ATATGGCCAG	TGTGCCGGTC	TCCGTTATCG	GGGAAGAAGT	GGCTGATCTC	AGCCACCGCG
601	AAAATGACAT	CAAAAACGCC	ATTAACCTGA	TGTTCTGGGG	AATATAGAAT	TCGCGGCCGC
661	ACTCGAGATA	TCTAGACCCA	GCTTTCTTGT	ACAAAGTTGG	CATTATAAGA	AAGCATTGCT
721	TATCAATTTG	TTGCAACGAA	CAGGTCACCT	TCAGTCAAAA	TAAATCATT	ATTTGCCATC
781	CAGCTGCAGC	TCTGGCCCGT	GTCTCAAAAT	CTCTGATGTT	ACATTGCACA	AGATAAAAAAT
841	ATATCATCAT	GAACAATAAA	ACTGCTGTCT	TACATAAACA	GTAATACAAG	GGGTGTTATG
901	AGCCATATTC	AACGGGAAAC	GTCGAGGCCG	CGATTAAATT	CCAACATGGA	TGCTGATTTA
961	TATGGGTATA	AATGGGCTCG	CGATAATGTC	GGGCAATCAG	GTGCGACAAT	CTATCGCTTG
1021	TATGGGAAAGC	CCGATGCGCC	AGAGTTGTTT	CTGAAACATG	GCAAAGGTAG	CGTTGCCAAT
1081	GATGTTACAG	ATGAGATGGT	CAGACTAAAC	TGGCTGACGG	AATTTATGCC	TCTTCCGACC
1141	ATCAAGCATT	TTATCCGTAC	TCCTGATGAT	GCATGGTTAC	TCACCACTGC	GATCCCCGGA
1201	AAACACAGCAT	TCCAGGTATT	AGAAGAATAT	CCTGATTTCAG	GTGAAAATAT	TGTTGATGCG
1261	CTGGCAGTGT	TCCTGCGCCG	GTTGCATTCT	ATTCTGTTT	GTAATTGTCC	TTTTAACAGC
1321	GATCGCGTAT	TTCTGCTCGC	TCAGGCGCAA	TCACGAATGA	ATAACGGTTT	GGTTGATGCG
1381	AGTGATTTTG	ATGACGAGCG	TAATGGCTGG	CCTGTTGAAC	AAGTCTGGAA	AGAAATGCAT
1441	AAACTTTTTC	CATTCTCACC	GGATTTCAGT	GTCACCTCAT	GTGATTTCTC	ACTTGATAAC
1501	CTTATTTTTC	ACGAGGGGAA	ATTAATAGGT	TGTATTGATG	TTGGACGAGT	CGGAATCGCA
1561	GACCGATACC	AGGATCTTGC	CATCCATATG	AACTGCCTCG	GTGAGTTTTT	TCCTTCATTA
1621	CAGAAACGGC	TTTTTCAAAA	ATATGGTATT	GATAATCCTG	ATATGAATAA	ATTGCAGTTT
1681	CATTTGATGC	TCGATGAGTT	TTTCTAATCA	GAATTGGTTA	ATTGGTTGTA	ACATTATTCA
1741	GATTGGGCC	CGTTCCACTG	AGCGTCAGAC	CCCGTAGAAA	AGATCAAAGG	ATCTTCTTGA
1801	GATCCTTTT	TTCTGCGCGT	AATCTGCTGC	TTGCAAACAA	AAAAACCACC	GCTACCAGCG
1861	GTGGTTTGT	TGCCGGATCA	AGAGCTACCA	ACTCTTTTTC	CGAAGGTAAC	TGGCTTCAGC
1921	AGAGCGCAGA	TACCAAATAC	TGTTCTTCTA	GTGTAGCCGT	AGTTAGGCCA	CCACTTCAAG
1981	AACTCTGTAG	CACCGCCTAC	ATACCTCGCT	CTGCTAATCC	TGTTACCAGT	GGCTGCTGCC
2041	AGTGGCGATA	AGTCGTGTCT	TACCGGGTTG	GACTCAAGAC	GATAGTTACC	GGATAAGGCG
2101	CAGCGGTCCG	GCTGAACGGG	GGGTTTCGTG	ACACAGCCCA	GCTTGAGAGC	AACGACCTAC
2161	ACCGAATCTG	GATACCTACA	GCGTGAGCTA	TGAGAAAGCG	CCACGCTTCC	CGAAGGGAGA
2221	AAGGCGGACA	GGTATCCGGT	AAGCGGCAGG	GTCGGAACAG	GAGAGCGCAC	GAGGGAGCTT
2281	CCAGGGGGAA	ACGCCTGGTA	TCTTTATAGT	CCTGTCGGGT	TTCCGCCACT	CTGACTTGAG
2341	CGTCGATTTT	TGTGATGCTC	GTCAGGGGGG	CGGAGCCTAT	GGAAAAACGC	CAGCAACGCG
2401	GCCTTTTTTAC	GGTTCCTGGC	CTTTTGCTGG	CCTTTTGCTC	ACATGTTCTT	TCCTGCGTTA
2461	TCCCCTGATT	CTGTGGATAA	CCGTATTACC	GCTAGCATGG	ATCTCGGGGA	CGTCTAACTA
2521	CTAAGCGAGA	GTAGGGAAC	GCCAGGCATC	GAATAAAACG	AAAGGCTCAG	TCGGAAGACT
2581	GGGCCTTTTC	TTTTATCTGT	TGTTTGTCGG	TGAACGCTCT	CCTGAGTAGG	ACAAATCCGC
2641	CGGGAGCGGA	TTTGAACGTT	GTGAAGCAAC	GGCCCGGAGG	GTGGCGGGCA	GGACGCCCGC
2701	CATAAACTGC	CAGGCATCAA	ACTAAGCAGA	AGGCCATC		

FIGURE 19B

Kozak NcoI SalI BamHI KpnI EcoRI EcoRI NotI

TTA ACC ATG GTC GAC TGG ATC CGG TAC CGA ATT C-- ccdB --G AAT TCG CGG CCG  
AAT TGG TAC CAG CTG ACC TAG GGC ATG GCT TAA G C TTA AGC GCC GGC

Leu Thr Met Val Asp Trp Ile Arg Tyr Arg Ile Asn Ser Arg Pro

XhoI            EcoRV   XbaI                            Int<sup>r</sup>   attL2

CAC TCG AGA TAT CTA GAC CCA GCT TTC TTG TAC AAA G  
GTG AGC TCT ATA GAT CTG GGT CGA AAG AAC ATG TTT C

His Ser Arg Tyr Leu Asp Pro Ala Phe Leu Tyr Lys

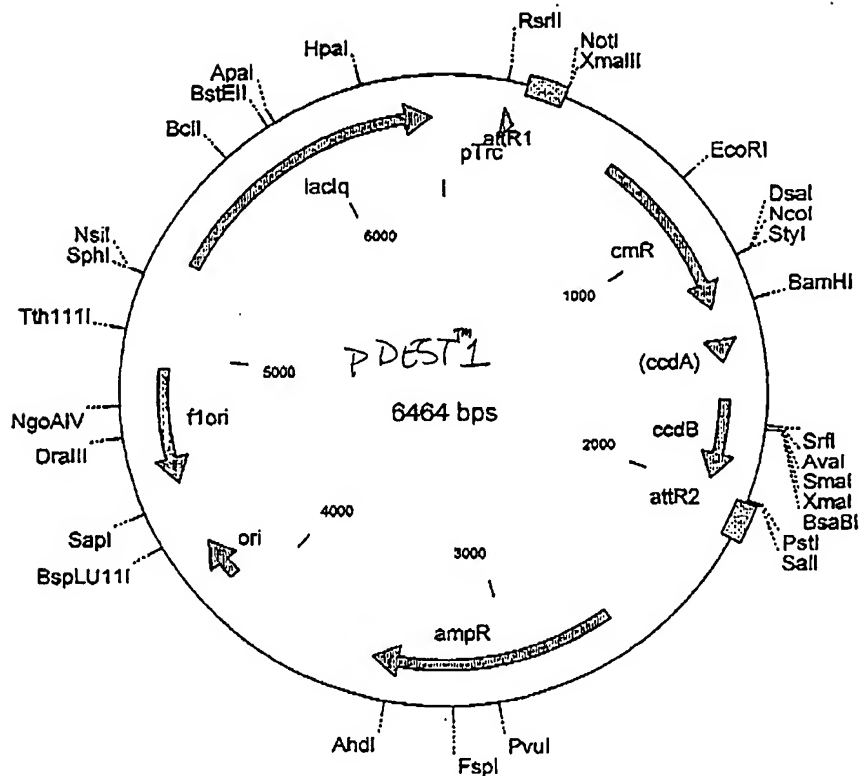
## pENTR11 2744 bp (rotated to position 2578)

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
67..166		attL1
348..653		ccdB
683..781		attL2
904..1713		KmR
1818..2391		ori
1	CTGACGGATG GCCTTTTTCG GTTTCTACAA ACTCTTCTCG TTAGTTAGTT ACTTAAGCTC	
61	GGGCCCCAAA TAATGATTTT ATTTTGACTG ATAGTGACCT GTTCGTTGCA ACAAATTGAT	
121	AAGCAATGCT TTTTATAAAT GCCAACTTTG TACAAAAAAG CAGGCTTCGA AGGAGATAGA	
181	ACCAATTCTC TAAGGAAATA CTTAACCATG GTCGACTGGA TCCGGTACCG AATTCGCTTA	
241	CTAAAAGCCA GATAACAGTA TGCCTATTTG CGCGCTGATT TTGCGGTAT AAGAATATAT	
301	ACTGATATGT ATACCCGAAG TATGTCAAAA AGAGGTGTGC TTCTAGAATG CAGTTTAAGG	
361	TTTACACCTA TAAAAGAGAG AGCCGTTATC GTCTGTTTGT GGATGTACAG AGTGATATTA	
421	TTGACACGCC CGGGCGACGG ATAGTGATCC CCCTGGCCAG TGCACGTCTG CTGTCAGATA	
481	AAGTCTCCCG TGAACCTTAC CCGGTGGTGC ATATCGGGGA TGAAAGCTGG CGCATGATGA	
541	CCACCGATAT GGCCAGTGTG CCGGTCTCCG TTATCGGGGA AGAAGTGGCT GATCTCAGCC	
601	ACCGCGAAAA TGACATCAAA AACGCCATTA ACCTGATGTT CTGGGGAATA TAGAATTCGC	
661	GGCCGCACTC GAGATATCTA GACCCAGCTT TCTTGATCAA AGTTGGCATT ATAAGAAAGC	
721	ATTGCTTATC AATTTGTTGC AACGAACAGG TCACTATCAG TCAAAATAAA ATCATTATTT	
781	GCCATCCAGC TGCAGCTCTG GCCCGTGTCT CAAAATCTCT GATGTTACAT TGCACAAGAT	
841	AAAAATATAT CATCATGAAC AATAAAACTG TCTGCTTACA TAAACAGTAA TACAAGGGGT	
901	GTTATGAGCC ATATTCAACG GGAAACGTCG AGGCCGCGAT TAAATCCCAA CATGGATGCT	
961	GATTTATATG GGTATAAATG GGCTCGCGAT AATGTCGGGC AATCAGGTGC GACAATCTAT	
1021	CGCTTGTATG GGAAGCCCCG TGCGCCAGAG TTGTTTCTGA AACATGGCAA AGGTAGCGTT	
1081	GCCAATGATG TTACAGATGA GATGGTCAGA CTAAACTGGC TGACGGAATT TATGCCTCTT	
1141	CCGACCATCA AGCATTTTAT CCGTACTCCT GATGATGCAT GGTACTCAC CACTGCGATC	
1201	CCCGGAAAAA CAGCATTCCA GGTATTAGAA GAATATCCTG ATTCAGGTGA AAATATTGTT	
1261	GATGCGCTGG CAGTGTTTCT GCGCCGGTTG CATTGCTTTC CTGTTTGTA TTTGCTCTTT	
1321	AACAGCGATC GCGTATTTCT TCTCGCTCAG GCGCAATCAC GAATGAATA CGGTTTGTT	
1381	GATGCGAGTG ATTTTGATGA CGAGCGTAAT GGCTGCGCTG TTGAACAAGT CTGGAAGAA	
1441	ATGCATAAAC TTTTGCCATT CTCACCGGAT TCAGTCGTCA CTCATGGTGA TTTCTCACTT	
1501	GATAACCTTA TTTTGACGA GGGGAAATTA ATAGGTTGTA TTGATGTTGG ACGAGTCGGA	
1561	ATCGCAGACC GATACCAGGA TCTTGCCATC CTATGGAAC GCCTCGGTGA GTTTTCTCCT	
1621	TCATTACAGA AACGGCTTTT TCAAAAATAT GGTATTGATA ATCCTGATAT GAATAAATTG	
1681	CAGTTTCATT TGATGCTCGA TGAGTTTTC TAATCAGAAT TGGTTAATTG GTTGTAACAT	
1741	TATTCAGATT GGGCCCCGTT CCACTGAGCG TCAGACCCCG TAGAAAAGAT CAAAGGATCT	
1801	TCTTGAGATC CTTTTTTTCT GCGCGTAATC TGCTGCTTGC AAACAAAAAA ACCACCGCTA	
1861	CCAGCGGTGG TTTGTTTGCC GGATCAAGAG CTACCAACTC TTTTCCGAA GGTAAGTGGC	
1921	TTCAGCAGAG CGCAGATACC AAATACTGTT CTTCTAGTGT AGCCGTAGTT AGGCCACCAC	
1981	TTCAAGAACT CTGTAGCACC GCCTACATAC CTCGCTCTGC TAATCCTGTT ACCAGTGGCT	
2041	GCTGCCAGTG GCGATAAGTC GTGTCTTACC GGGTTGGACT CAAGACGATA GTTACCGGAT	
2101	AAGGCGCAGC GGTGCGGCTG AACGGGGGGT TCGTGACAC AGCCAGCTT GGAGCGAAGC	
2161	ACCTACACCG AACTGAGATA CCTACAGCGT GAGCTATGAG AAAGCGCCAC GCTTCCCGAA	
2221	GGGAGAAAGG CGGACAGGTA TCCGGTAAGC GGCAGGGTCG GAACAGGAGA GCGCACGAGG	
2281	GAGCTTCCAG GGGGAAACGC CTGGTATCTT TATAGTCTCTG TCGGGTTTCG CCACCTCTGA	
2341	CTTGAGCGTC GATTTTTGTG ATGCTCGTCA GGGGGGCGGA GCCTATGGAA AAACGCCAGC	
2401	AACGCGGCCT TTTACGGTT CCTGGCCTT TGCTGGCCTT TTGCTCACAT GTTCTTTCTT	
2461	GCGTTATCCC CTGATTCTGT GGATAACCGT ATTACCGCTA GCATGGATCT CGGGGAGCTC	
2521	TAATACTAA GCGAGAGTAG GGAAGTGCCA GGCATCAAAT AAAACGAAAG GCTCAGTCGG	
2581	AAGACTGGGC CTTTCGTTT ATCTGTTGTT TGTCGGTGAA CGCTCTCTCTG AGTAGGACAA	
2641	ATCCGCCGGG AGCGGATTG AACGTTGTGA AGCAACGGCC CGGAGGGTGG CGGGCAGGAC	
2701	GCCCGCCATA AACTGCCAGG CATCAAATA AGCAGAAGGC CATC	

FIGURE 20B

Figure 2/A: pDEST1 Native Protein Expression in E. coli

1 atgagctg <sup>-35</sup> gacaattaat <sup>Tre promoter</sup> catccggctc <sup>-10</sup> gataatgtg <sup>RNA</sup> tggattgtg agcggataac  
 tactcgaca ctgttaatta gtaggccgag catattacac accttaacac tcgcctattg  
 61 aatttcacac aggaacaga caggtatagg atcacaagtt gttgaadaa <sup>attR1</sup> agctgaagga  
 ttaaagtgtg tcccttgtct gtccatatec tag gttcaa acatgtttt tcgactcgt



33/240

## pDEST1 6464 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
216..257		Trc promoter
397..273		attR1
647..1306		CmR
1426..1510		inactivated ccdA
1648..1953		ccdB
1994..2118		attR2
2598..3503		ampR
4104..4264		ori
4504..4941		flori (f1 intergenic region)
5340..6420		lacIq

1	GTTTGACAGC	TTATCATCGA	CTGCACGGTG	CACCAATGCT	TCTGGCGTCA	GGCAGCCATC
61	GGAAGCTGTG	GTATGGCTGT	GCAGGTCGTA	AATCACTGCA	TAATTCGTGT	CGCTCAAGGC
121	GCACTCCCGT	TCTGGATAAT	GTTTTTTGCG	CCGACATCAT	AACGGTTCTG	GCAAATATTC
181	TGAAATGAGC	TGTTGACAAT	TAATCATCCG	GTCCGTATAA	TCTGTGGAAT	TGTGAGCGGG
241	ATAACAATTT	CATCGCGAGG	TACCAAGCTA	TCACAAGTTT	GTACAAAAAA	GCTGAACGAG
301	AAACGTAAAA	TGATATAAAT	ATCAATATAT	TAAATTAGAT	TTTGCATAAA	AAACAGACTA
361	CATAATACTG	TAAAACACAA	CATATCCAGT	CACTATGGCG	GCCGCTAAGT	TGGCAGCATC
421	ACCCGACGCA	CTTTGCGCCG	AATAAATACC	TGTGACGGAA	GATCACTTCG	CAGAATAAAT
481	AAATCCTGGT	GTCCCTGTTG	ATACCGGGAA	GCCCTGGGCC	AACTTTTGGC	GAAAATGAGA
541	CGTTGATCGG	CACGTAAGAG	GTTCCAACCT	TCACCATAAT	GAAATAAGAT	CACTACCGGG
601	CGTATTTT	GAGTTATCGA	GATTTTCAGG	AGCTAAGGAA	GCTAAAATGG	AGAAAAAAT
661	CACTGGATAT	ACCACCGTTG	ATATATCCCA	ATGGCATCGT	AAAGAACATT	TTGAGGCATT
721	TCAGTCAGTT	GCTCAATGTA	CCTATAACCA	GACCGTTCAG	CTGGATATTA	CGGCCTTTTT
781	AAAGACCGTA	AAGAAAAATA	AGCACAAGTT	TTATCCGGCC	TTTATTACCA	TTCTTGCCCC
841	CCTGATGAAT	GCTCATCCGG	AATTCCGTAT	GGCAATGAAA	GACGGTGAGC	TGGTGATATG
901	GGATAGTGTT	CACCCCTGTT	ACACCGTTTT	CCATGAGCAA	ACTGAAACGT	TTTCATCGCT
961	CTGGAGTGAA	TACCACGACG	ATTTCCGGCA	GTTTCTACAC	ATATATTTCG	AAGATGTGGC
1021	GTGTTACGGT	GAAAACCTGG	CCTATTTCCC	TAAAGGGTTT	ATTGAGAATA	TGTTTTTCGT
1081	CTCAGCCAAT	CCCTGGGTGA	GTTTCACCAG	TTTTGATTTA	AACGTGGCCA	ATATGGACAA
1141	CTTCTTCGCC	CCCGTTTTCA	CCATGGGCAA	ATATTATACG	CAAGGCGACA	AGGTGCTGAT
1201	GCCGCTGGCG	ATTGAGGTTT	ATCATGCCGT	CTGTGATGGC	TTCCATGTCT	GCAGAAATGCT
1261	TAATGAATTA	CAACAGTACT	GCGATGAGTG	GCAGGGCGGG	GCGTAAACGC	GTGGATCCGG
1321	CTTACTAAAA	GCCAGATAAC	AGTATGCGTA	TTTGCGCGCT	GATTTTTGCG	GTATAAGSAAT
1381	ATATACTGAT	ATGTATACCC	GAAGTATGTC	AAAAAGAGGT	GTGCTATGAA	GCAGCGTATT
1441	ACAGTGACAG	TTGACAGCGA	CAGCTATCAG	TTGCTCAAGG	CATATATGAT	GTCAATATCT
1501	CCGGTCTGGT	AAGCACAACC	ATGCAGAATG	AAGCCCGTCG	TCTGCGTGCC	GAACGCTGGA
1561	AAGCGGAAAA	TCAGGAAGGG	ATGGCTGAGG	TCGCCCGGTT	TATTGAAATG	AACGGCTCTT
1621	TTGCTGACGA	GAACAGGGAC	TGGTGAAATG	CAGTTTAAAG	TTTACACCTA	TAAAAGAGAG
1681	AGCCGTTATC	GTCTGTTTGT	GGATGTACAG	AGTGATATTA	TTGACACGCC	CGGGCGACGG
1741	ATGGTGATCC	CCCTGGCCAG	TGCACGTCTG	CTGTCTAGATA	AAGTCTCCCG	TGAACCTTAC
1801	CCGGTGGTGC	ATATCGGGGA	TGAAAGCTGG	CGCATGATGA	CCACCGATAT	GGCCAGTGTG
1861	CCGGTCTCCG	TTATCGGGGA	AGAAGTGGCT	GATCTCAGCC	ACCGCGAAAA	TGACATCAAA
1921	AACGCCATTA	ACCTGATGTT	CTGGGGAATA	TAAATGTCAG	GCTCCCTTAT	ACACAGCCAG
1981	TCTGCAGGTC	GACCATAGTG	ACTGGATATG	TTGTGTTTTA	CAGTATTATG	TAGTCTGTTT
2041	TTTATGCAAA	ATCTAATTTA	ATATATTGAT	ATTTATATCA	TTTTACGTTT	CTCGTTCAGC
2101	TTTTCTGTAC	AAAGTGGTGA	TAGCTTGGCT	GTTTTGGCGG	ATGAGAGAAG	ATTTTCAGCC
2161	TGATACAGAT	TAAATCAGAA	CGCAGAAGCG	GTCTGATAAA	ACAGAATTTG	CCTGGCGGCA
2221	GTAGCGCGGT	GGTCCCACCT	GACCCCATGC	CGAACTCAGA	AGTGAAACGC	CGTAGCGCCG
2281	ATGGTAGTGT	GGGGTCTCCC	CATGCGAGAG	TAGGGAACTG	CCAGGCATCA	AATAAAACGA
2341	AAGGCTCAGT	CGAAAGACTG	GGCCTTTCGT	TTTATCTGTT	GTTTGTCTGGT	GAACGCTCTC
2401	CTGAGTAGGA	CAAAATCCGCC	GGGAGCGGAT	TTGAACGTTG	CGAAGCAACG	GCCCCGAGGG
2461	TGGCGGGCAG	GACGCCCCGC	ATAAACTGCC	AGGCATCAAA	TTAAGCAGAA	GGCCATCCTG
2521	ACGGATGGCC	TTTTTGCCTT	TCTACAAACT	CTTTTTGTTT	ATTTTTCTAA	ATACATTCAA-

FIGURE 21B

34/240

```

2581 ATATGTATCC GCTCATGAGA CAATAACCCT GATAAATGCT TCAATAATAT TGAAAAAGGA
2641 AGAGTATGAG TATTCAACAT TTCCGTGTCTG CCCTTATTCC CTTTTTTGCG GCATTTTGCC
2701 TTCCTGTTTT TGCTCACCCA GAAACGCTGG TGAAAGTAAA AGATGCTGAA GATCAGTTGG
2761 GTGCACGAGT GGGTTACATC GAACTGGATC TCAACAGCGG TAAGATCCTT GAGAGTTTTT
2821 GCCCCGAAGA ACGTTTTCCA ATGATGAGCA CTTTTAAAGT TCTGCTATGT GGCGCGGTAT
2881 TATCCCGTGT TGACGCGGGG CAAGAGCAAC TCGGTCTGCC CATACACTAT TCTCAGAATG
2941 ACTTGGTTGA GTACTACCA GTCACAGAAA AGCATCTTAC GGATGGCATG ACAGTAAGAG
3001 AATTATGCAG TGCTGCCATA ACCATGAGTG ATAACACTGC GGCCAACTTA CTTCTGACAA
3061 CGATCGGAGG ACCGAAGGAG CTAACCGCTT TTTTGACAA CATGGGGGAT CATGTAATC
3121 GCCTTGATCG TTGGGAACCG GAGCTGAATG AAGCCATACC AAACGACGAG CGTGACACCA
3181 CGATGCCTAC AGCAATGGCA ACAACGTTGC GCAAACCTATT AACTGGCGAA CTACTTACTC
3241 TAGCTTCCCG GCAACAATTA ATAGACTGGA TGGAGGCGGA TAAAGTTGCA GTGCCACTTC
3301 TGCGCTCGGC CCTTCCGGCT GGCTGGTTTA TTGCTGATAA ATCTGGAGCC GGTGAGCGTG
3361 GGTCTCGCGG TATCATTGCA GCACTGGGGC CAGATGGTAA GCCCTCCCGT ATCGTAGTTA
3421 TCTACACGAC GGGGAGTCAG GCAACTATGG ATGAACGAAA TAGACAGATC GCTGAGATAG
3481 GTGCCTCACT GATTAAGCAT TGGTAACTGT CAGACCAAGT TTAATCATAT ATACTTTAGA
3541 TTGATTTAAA ACTTCATTTT TAATTTAAAA GGATCTAGGT GAAGATCCTT TTTGATAATC
3601 TCATGACCAA AATCCCTTAA CGTGAGTTTT CGTTCCACTG AGCGTCAGAC CCCGTAGAAA
3661 AGATCAAAGG ATCTTCTTGA GATCCTTTTT TTCTGCGCGT AATCTGCTGC TTGCAAAACA
3721 AAAAACCACC GCTACCAGCG GTGGTTTGTT TGCCGGATCA AGAGCTACCA ACTCTTTTTT
3781 CGAAGGTAAC TGGCTTCAGC AGAGCGCAGA TACCAAATAC TGTCCTTCTA GTGTAGCCGT
3841 AGTTAGGCCA CCACTTCAAG AACTCTGTAG CACCGCTAC ATACCTCGCT CTGCTAATCC
3901 TGTTACCAGT GGCTGCTGCC AGTGGCGATA AGTCGTGTCT TACCGGGTTG GACTCAAGAC
3961 GATAGTTACC GGATAAGGCG CAGCGGTCCG GCTGAACGGG GGGTTCGTGC ACACAGCCCA
4021 GCTTGGAGCG AACGACCTAC ACCGAACCTGA GATACCTACA GCGTGAGCTA TGAGAAAGCG
4081 CCACGCTTCC CGAAGGGAGA AAGGCGGACA GGTATCCGGT AAGCGGCAGG GTCGGAACAG
4141 GAGAGCGCAC GAGGGAGCTT CCAGGGGGAA ACGCCTGGTA TCTTTATAGT CCTGTGCGGT
4201 TTCGCCACCT CTGACTTGAG CGTCGATTTT TGTGATGCTC GTCAGGGGGG CGGAGCCTAT
4261 GAAAAACGCG CAGCAACGCG GCCTTTTTTAC GGTTCCTGGC CTTTTGCTGG CTTTTTGCTC
4321 ACATGTTCTT TCCTGCGTTA TCCCCTGATT CTGTGGATAA CCGTATTACC GCCTTTGAGT
4381 GAGCTGATAC CGCTCGCCGC AGCCGAACGA CCGAGCGCAG CGAGTCAGTG AGCGAGGAAG
4441 CGGAAGAGCG CCTGATGCGG TATTTCTTCC TTACGCATCT GTGCGGTATT TCACACCGCA
4501 TAATTTTGTT AAAATTCGCG TTAAATTTTT GTTAAATCAG CTCATTTTTT AACCAATAGG
4561 CCGAAATCGG CAAAATCCCT TATAAATCAA AAGAATAGAC CGAGATAGGG TTGAGTGTTG
4621 TTCCAGTTTG GAACAAGAGT CCACTATTAA AGAACGTGGA CTCCAACGTC AAAGGGCGAA
4681 AAACCGTCTA TCAGGGCGAT GGCCCACTAC GTGAACCATC ACCCTAATCA AGTTTTTTGT
4741 GGTGCGAGTG CCGTAAAGCA CTAATTCGGA ACCCTAAAGG GAGCCCCGTA TTTAGAGCTT
4801 GACGGGGAAA GCCGGCGAAC GTGGCGAGAA AGGAAGGGAA GAAAGCGAAA GGAGCGGGCG
4861 CTAGGGCGCT GGCAAGTGTA GCGGTCACGC TGCGCGTAAC CACCACACCC GCCGCGCTTA
4921 ATGCGCCGCT ACAGGGCGCG TCCATTGCGC ATTACAGGCTG CTATGGTGCA CTCTCAGTAC
4981 AATCTGCTCT GATGCCGCAT AGTTAAGCCA GTACCAGTCA CGTAGCGATA TCGAGTGTA
5041 TACACTCCGC TATCGCTACG TGACTGGGTC ATGGCTGCGC CCCGACACCC GCCAACACCC
5101 GCTGACGCGC CCTGACGGGC TTGTCTGCTC CCGCATCCG CTTACAGACA AGCTGTGACC
5161 GTCTCCGGGA GCTGCATGTG TCAGAGGTTT TCACCGTCAT CACCGAAACG CGCGAGGCAG
5221 CAGATCAATT CGCGCGCGAA GGCGAAGCGG CATGCATTTA CGTTGACACC ATCGAATGGT
5281 GCAAAACCTT TCGCGGTATG GCATGATAGC GCCCGGAAGA GAGTCAATTC AGGGTGGTGA
5341 ATGTGAAACC AGTAACGTTA TACGATGTCG CAGAGTATGC CGGTGTCTCT TATCAGACCG
5401 TTTCCGCGT GGTGAACCA GGCAGCCACG TTTCTGCGAA AACGCGGGAA AAAGTGAAG
5461 CGGCGATGGC GGAGCTGAAT TACATTCCCA ACCGCGTGGC ACAACAATG GCGGGCAAC
5521 AGTCGTTGCT GATTGGCGTT GCCACCTCCA GTCTGGCCCT GCACGCGCGG TCGCAAATTG
5581 TCGCGGCGAT TAAATCTCGC GCCGATCAAC TGGGTGCCAG CGTGGTGGTG TCGATGGTAG
5641 AACGAAGCGG CGTCGAAGCC GTAAAGCGG CGGTGCACAA TCTTCTCGCG CAACGCGTCA
5701 GTGGGCTGAT CATTAACTAT CCGCTGGATG ACCAGGATGC CATTGCTGTG GAAGCTGCCT
5761 GCACATAATGT TCCGGCGTTA TTTCTTGATG TCTCTGACCA GACACCATC AACAGTATTA
5821 TTTTCTCCCA TGAAGACGGT ACGCGACTGG GCGTGGAGCA TCTGGTCGCA TTGGTCAAC
5881 AGCAAATCGC GCTGTTAGCG GGCCCATTA GTTCTGTCTC GGCGCGTCTG CGTCTGGCTG
5941 GCTGGCATAA ATATCTCACT CGCAATCAAA TTCAGCCGAT AGCGGAACCG GAAGCGGACT
6001 GGAGTGCCAT GTCCGGTTTT CAACAAACCA TGCAAATGCT GAATGAGGGC ATCGTTCCCA-

```

FIGURE 21C



35/240

6061 CTGCGATGCT GGTGCGCAAC GATCAGATGG CGCTGGGCGC AATGCGCGCC ATTACCGAGT  
6121 CCGGGCTGCG CGTTGGTGCG GATATCTCGG TAGTGGGATA CGACGATACC GAAGACAGCT  
6181 CATGTTATAT CCCGCCGTTA ACCACCATCA AACAGGATTT TCGCCTGCTG GGGCAAACCA  
6241 GCGTGGACCG CTGCTGCAA CTCTCTCAGG GCCAGGCGGT GAAGGGCAAT CAGCTGTTGC  
6301 CCGTCTCACT GGTGAAAAGA AAAACCAACC TGGCACCCAA TACGCAAACC GCCTCTCCCC  
6361 GCGCGTTGGC CGATTCATTA ATGCAGCTGG CACGACAGGT TTCCCGACTG GAAAGCGGGC  
6421 AGTGAGCGCA ACGCAATTAA TGTGAGTTAG CGCGAATTGA TCTG

FIGURE 21D

36/240

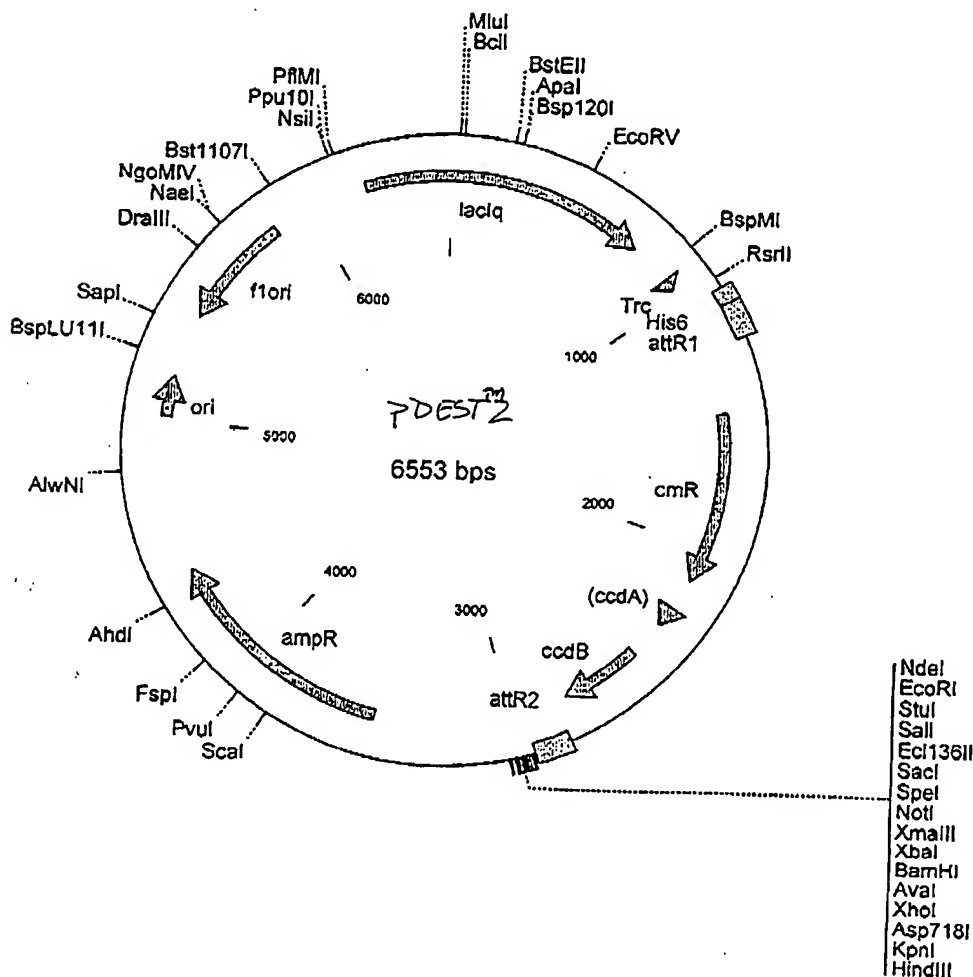
Figure 22A: pDEST2

His6 fusions in E. coli

970 aat att ctg aaa tga gct <sup>-35</sup> gtt gac aat taa tca tcc ggt ccg <sup>-10</sup> tat aat ctg  
 tta taa gac ttt act cga aaa ctg tta att agt agg cca ggc ata tta gac

1021 tgg <sup>→ RNA</sup> aat tgt gag cgg ata aca att tca cac agg aaa cag acc atg tcg <sup>Met Ser Tyr</sup> ttc  
 acc tta aca ctc gcc tat tgt taa agt gtg tcc ttt gtc tgg tac agc atg

1072 <sup>Tyr His His His His His His</sup> tac cat cac cat cac cat cat <sup>Glu Ile</sup> ggt atc <sup>Trp Ser Tyr</sup> aca agt tgg taa <sup>attR1</sup> aaa gcy gaa  
 atg gta gtg gta gtg gta gtg ccg tag tgt tca aac atg ttt ctt cga cxt



37/240

## pDEST2 6553 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
912..962		Trc
1223..1009		attR1
1473..2132		CmR
2252..2336		inactivated ccdA
2474..2779		ccdB
2820..2944		attR2
3509..4414		ampR
5015..5175		ori
5415..5852		flori (f1 intergenic region)
6225..752		lacIq

1	GGCGGTGCAC	AATCTTCTCG	CGCAACGCGT	CAGTGGGCTG	ATCATTAACT	ATCCGCTGGA
61	TGACCAGGAT	GCCATTGCTG	TGGAAGCTGC	CTGCACTAAT	GTTCCGGCGT	TATTTCTTGA
121	TGTCTCTGAC	CAGACACCCA	TCAACAGTAT	TATTTTCTCC	CATGAAGACG	GTACGCGACT
181	GGGCGTGGAG	CATCTGGTGC	CATTGGGTCA	CCAGCAAATC	GCGCTGTTAG	CGGGCCCATT
241	AAGTTCTGTC	TCGGCGCGTC	TGCGTCTGGC	TGGCTGGCAT	AAATATCTCA	CTCGCAATCA
301	AATTCAGCCG	ATAGCGGAAC	GGGAAGGCGA	CTGGAGTGCC	ATGTCCGGTT	TTCAACAAAC
361	CATGCAAATG	CTGAATGAGG	GCATCGTTCC	CACTGCGATG	CTGGTTGCCA	ACGATCAGAT
421	GGCGCTGGGC	GCAATGCGCG	CCATTACCGA	GTCCGGGCTG	CGCGTTGGTG	CGGATATCTC
481	GGTAGTGGGA	TACGACGATA	CCGAAGACAG	CTCATGTTAT	ATCCCGCCGT	CAACCACCAT
541	CAAACAGGAT	TTTCGCCTGC	TGGGGCAAAC	CAGCGTGGAC	CGCTTGCTGC	AACTCTCTCA
601	GGGCCAGGCG	GTGAAGGGCA	ATCAGCTGTT	GCCCGTCTCA	CTGGTGAAAA	GA AAAAACAC
661	CCTGGCACCC	AATACGCAAA	CCGCCTCTCC	CCGCGCGTTG	GCCGATTCAT	TAATGCAGCT
721	GGCACGACAG	GTTTCCCGAC	TGGAAGCGG	GCAGTGAGCG	CAACGCAATT	AATGTGAGTT
781	AGCGCGAATT	GATCTGGTTT	GACAGCTTAT	CATCGACTGC	ACGGTGCACC	AATGCTTCTG
841	GCGTCAGGCA	GCCATCGGAA	GCTGTGGTAT	GGCTGTGCAG	GTCTGAAATC	ACTGCATAAT
901	TCGTGTCGCT	CAAGGCGCAC	TCCCGTTCTG	GATAATGTTT	TTTGCGCCGA	CATCATAACG
961	GTTCTGGCAA	ATATTCTGAA	ATGAGCTGTT	GACAATTAAT	CATCCGGTCC	GTATAATCTG
1021	TGGAATTGTG	AGCGGATAAC	AATTTACAC	AGGAAACAGA	CCATGTCGTA	CTACCATCAC
1081	CATCACCATC	ACGGCATCAC	AAGTTTGTAC	AAAAAAGCTG	AACGAGAAAC	GTAAAATGAT
1141	ATAAATATCA	ATATATTAAT	TTAGATTTTG	CATAAAAAAC	AGACTACATA	ATACTGTAAA
1201	ACACAACATA	TCCAGTCACT	ATGGCGGCCG	CTAAGTTGGC	AGCATCACCC	GACGCACTTT
1261	GCGCCGAATA	AATACCTGTG	ACGGAAGATC	ACTTCGCAGA	ATAAATAAAT	CCTGGTGTC
1321	CTGTTGATAC	CGGGAAGCCC	TGGGCCAACT	TTTGGCGAAA	ATGAGACGTT	GATCGGCACG
1381	TAGAGAGTTC	CAACTTTCAC	CATAATGAAA	TAAGATCACT	ACCGGGCGTA	TTTTTTGAGT
1441	TATCGAGATT	TTCAGGAGCT	AAGGAAGCTA	AAATGGAGAA	AAAAATCACT	GGATATACCA
1501	CCGTTGATAT	ATCCCAATGG	CATCGTAAAG	AACATTTTGA	GGCATTTTCA	TCAGTTGCTC
1561	AATGTACCTA	TAACCAGACC	GTTTCAGCTG	ATATTACGGC	CTTTTAAAG	ACCGTAAAGA
1621	AAAATAAGCA	CAAGTTTTAT	CCGGCCTTTA	TTTACATTCT	TGCCCGCCTG	ATGAATGCTC
1681	ATCCGGAATT	CCGTATGGCA	ATGAAAGACG	GTGAGCTGGT	GATATGGGAT	AGTGTTCACC
1741	CTTGTTACAC	CGTTTTCCAT	GAGCAAACCTG	AAACGTTTTT	ATCGCTCTGG	AGTGAATACC
1801	ACGACGATTT	CCGGCAGTTT	CTACACATAT	ATTCGCAAGA	TGTGGCGTGT	TACGGTGAAA
1861	ACCTGGCCTA	TTTCCCTAAA	GGGTTTATTG	AGAATATGTT	TTTCGTCTCA	GCCAATCCCT
1921	GGGTGAGTTT	CACCAAGTTT	GATTTAAACG	TGGCCAATAT	GGACAACCTT	TTCCGCCCCG
1981	TTTTACCAT	GGGCAAATAT	TATACGCAAG	GCGACAAGGT	GCTGATGCCG	CTGGCGATT
2041	AGGTTTCATCA	TGCCGTCTGT	GATGGCTTCC	ATGTCGGCAG	AATGCTTAAT	GAATTACAAC
2101	AGTACTGCGA	TGAGTGGCAG	GGCGGGCGGT	AAACGCGTGG	ATCCGGCTTA	CTAAAAGCCA
2161	GATAACAGTA	TGCGTATTTG	CGCGCTGATT	TTTGGCGTAT	AAGAATATAT	ACTGATATGT
2221	ATACCCGAAG	TATGTCAAAA	AGAGGTGTGC	TATGAAGCAG	CGTATTACAG	TGACAGTTGA
2281	CAGCGACAGC	TATCAGTTGC	TCAAGGCATA	TATGATGTCA	ATATCTCCGG	TCTGGTAAAG
2341	ACAACCATGC	AGAATGAAGC	CGTCTGCTCG	CGTGCCGAAC	GCTGGAAAGC	GGAAAATCAG
2401	GAAGGGATGG	CTGAGGTGCG	CCGCTTTATT	GAAATGAACG	GCTCTTTTGC	TGACGAGAAC
2461	AGGGACTGGT	GAAATGCAGT	TTAAGGTTTA	CACCTATAAA	AGAGAGAGCC	GTTATCGTCT
2521	GTTTGTGGAT	GTACAGAGTG	ATATTATTGA	CACGCCCGGG	CGACGGATGG	TGATCCCCCT-

FIGURE 22B

38/240

2581 GGCCAGTGCA CGTCTGCTGT CAGATAAAGT CTCCCGTGAA CTTTACCCGG TGGTGCAATAT  
2641 CGGGGATGAA AGCTGGCGCA TGATGACCAC CGATATGGCC AGTGTGCCGG TCTCCGTTAT  
2701 CGGGGAAGAA GTGGCTGATC TCAGCCACCG CGAAAATGAC ATCAAAAACG CCATTAACCT  
2761 GATGTTCTGG GGAATATAAA TGTCAGGCTC CCTTATACAC AGCCAGTCTG CAGGTCGACC  
2821 ATAGTGACTG GATATGTTGT GTTTTACAGT ATTATGTAGT CTGTTTTTTA TGCAAAATCT  
2881 AATTTAATAT ATTGATATTT ATATCATTTT ACGTTTCTCG TTCAGCTTTC TTGTACAAAG  
2941 TGGTGATGCC CATATGGGAA TTCAAAGGCC TACGTCGACG AGCTCACTAG TCGCGGCCGC  
3001 TTCTAGAGGA TCCCTCGAGG CATGCGGTAC CAAGCTTGGC TGTTTTGGCG GATGAGAGAA  
3061 GATTTTCAGC CTGATACAGA TTAATCAGA ACGCAGAAGC GGTCTGATAA AACAGAAATT  
3121 GCCTGGCGGC AGTAGCGCGG TGGTCCCACC TGACCCCATG CCGAACTCAG AAGTGAAACG  
3181 CCGTAGCGCC GATGGTAGTG TGGGGTCTCC CCATGCGAGA GTAGGGAACG GCCAGGCATC  
3241 AAATAAAACG AAAGGCTCAG TCGAAAGACT GGGCCTTTCG TTTTATCTGT TGTGTGTCGG  
3301 TGAACGCTCT CCTGAGTAGG ACAAATCCGC CGGGAGCGGA TTTGAACGTT GCGAAGCAAC  
3361 GGCCCGGAGG GTGGCGGGCA GGACGCCCGC CATAAACTGC CAGGCATCAA ATTAAGCAGA  
3421 AGGCCATCCT GACGGATGGC CTTTTTGCGT TTCTACAAAC TCTTTTTGTT TATTTTTCTA  
3481 AATACATTCA AATATGTATC CGCTCATGAG ACAATAACCC TGATAAATG TTCAATAATA  
3541 TTGAAAAAGG AAGAGTATGA GTATTCAACA TTTCCGTGTC GCCCTTATTC CCTTTTTTGC  
3601 GGCATTTTGC CTTCTGTTT TTGCTCACC AGAAACGCTG GTGAAAGTAA AAGATGCTGA  
3661 AGATCAGTTG GGTGCACGAG TGGGTACAT CGAACTGGAT CTCAACAGCG GTAAGATCCT  
3721 TGAGAGTTTT CGCCCCGAAG AACGTTTTCC AATGATGAGC ACTTTTAAAG TTCTGCTATG  
3781 TGGCGCGGTA TTATCCCGTG TTGACGCCGG GCAAGAGCAA CTCGGTCGCC GCATACACTA  
3841 TTCTCAGAAAT GACTTGGTTG AGTACTCACC AGTCACAGAA AAGCATCTTA CGGATGGCAT  
3901 GACAGTAAGA GAATTATGCA GTGCTGCCAT AACCATGAGT GATAACACTG CGGCCAACCT  
3961 ACTTCTGACA ACGATCGGAG GACCGAAGGA GCTAACCGCT TTTTTGCACA ACATGGGGGA  
4021 TCATGTAACCT CGCCTTGATC GTTGGGAACC GGAGCTGAAT GAAGCCATAC CAAACGACGA  
4081 GCGTGACACC ACGATGCCTA CAGCAATGGC AACAACGTTG CGCAAACAT TAACTGGCGA  
4141 ACTACTTACT CTAGCTTCCC GGCAACAATT AATAGACTGG ATGGAGGCGG ATAAAGTTGC  
4201 AGGACCACCT CTGCGCTCGG CCCTTCCGGC TGGCTGGTTT ATTGCTGATA AATCTGGAGC  
4261 CGGTGAGCGT GGGTCTCGCG GTATCATTGC AGCACTGGGG CCAGATGGTA AGCCCTCCCG  
4321 TATCGTAGTT ATCTACACGA CGGGGAGTCA GGCAACTATG GATGAACGAA ATAGACAGAT  
4381 CGCTGAGATA GGTGCCTCAC TGATTAAGCA TTGGTAACTG TCAGACCAAG TTTACTCATA  
4441 TATACTTTAG ATTGATTTAA AACTTCATTT TTAATTTAAA AGGATCTAGG TGAAGATCCT  
4501 TTTTGATAAT CTCATGACCA AAATCCCTTA ACGTGAGTTT TCGTTCCTACT GAGCGTCAGA  
4561 CCCCGTAGAA AAGATCAAAG GATCTTCTTG AGATCCTTTT TTTCTGCGCG TAATCTGCTG  
4621 CTTGCAAAACA AAAAAACCA CCGTACCAGC GGTGGTTTGT TTGCCGGATC AAGAGCTACC  
4681 AACTCTTTTT CCGAAGGTAA CTGGCTTCAG CAGAGCGCAG ATACCAAATA CTGTCCTTCT  
4741 AGTGAGCCG TAGTTAGGCC ACCACTTCAA GAACTCTGTA GCACCGCTA CATACTCGC  
4801 TCTGCTAATC CTGTTACCAG TGGCTGCTGC CAGTGGCGAT AAGTCGTGTC TTACCGGGT  
4861 GGACTIONA CGATAGTTAC CGGATAAGGC GCAGCGGTCG GGCTGAACGG GGGGTTCTGT  
4921 CACACAGCCC AGCTTGAGC GAACGACCTA CACCGAATG AGATACCTAC AGCGTGAGCT  
4981 ATGAGAAAGC GCCACGCTTC CCGAAGGGAG AAAGGCGGAC AGGTATCCGG TAAGCGGCAG  
5041 GGTGCGAACA GGAGAGCGCA CGAGGGAGCT TCCAGGGGGA AACGCCTGGT ATCTTTATAG  
5101 TCCTGTGCGG TTTGCGCAC TCTGACTTGA GCGTCGATTT TTGTGATGCT CGTCAGGGGG  
5161 GCGGAGCCTA TGGAAAAACG CCAGCAACGC GGCCTTTTTA CGGTTCCTGG CCTTTTGCTG  
5221 GCCTTTTGCT CACATGTTCT TTCCTGCGTT ATCCCCTGAT TCTGTGGATA ACCGTATTAC  
5281 CGCCTTTGAG TGAGCTGATA CCGCTCGCCG CAGCCGAACG ACCGAGCGCA GCGAGTCAGT  
5341 GAGCGAGGAA GCGGAAGAGC GCCTGATGCG GTATTTTCTC CTTACGCATC TGTGCGGTAT  
5401 TTCACACCGC ATAATTTTGT TAAAATTCGC GTTAAATTTT TGTTAAATCA GCTCATTTTT  
5461 TAACCAATAG GCCGAAATCG GCAAAATCCC TTATAAATCA AAAGAATAGA CCGAGATAGG  
5521 GTTGAGTGTT GTTCCAGTTT GGAACAAGAG TCCACTATTA AAGAACGTGG ACTCCAACGT  
5581 CAAAGGGCGA AAAACCGTCT ATCAGGCGCA TGGCCCACTA CGTGAACCAT CACCCTAATC  
5641 AAGTTTTTTG GGGTCGAGGT GCCGTAAAGC ACTAAATCGG AACCCTAAAG GGAGCCCCCG  
5701 ATTTAGAGCT TGACGGGAA AGCCGGCGAA CGTGGCGAGA AAGGAAGGGA AGAAAGCGAA  
5761 AGGAGCGGGC GCTAGGGCGC TGGCAAGTGT AGCGGTCACG CTGCGCGTAA CCACCACACC  
5821 CGCCGCGCTT AATGCGCCGC TACAGGGCGC GTCCCATTCG CCATTACAGG TGCTATGGTG  
5881 CACTCTCAGT ACAATCTGCT CTGATGCCGC ATAGTTAAGC CAGTATACAC TCCGCTATCG  
5941 CTACGTGACT GGGTCATGGC TGCGCCCCGA CACCCGCCAA CACCCGCTGA CGCGCCCTGA  
6001 CGGGCTTGTC TGCTCCCGGC ATCCGCTTAC AGACAAGCTG TGACCGTCTC CGGGAGCTGC

Figure 22C

39/240

6061 ATGTGTCAGA GGTTCACACC GTCATCACCG AAACGCGCGA GGCAGCAGAT CAATTCGCGC  
6121 GCGAAGGCGA AGCGGCATGC ATTTACGTTG ACACCATCGA ATGGTGCAAA ACCTTTCGCG  
6181 GTATGGCATG ATAGCGCCCG GAAGAGAGTC AATTCAGGGT GGTGAATGTG AAACCAGTAA  
6241 CGTTATACGA TGTCGCAGAG TATGCCGGTG TCTCTTATCA GACCGTTTCC CGCGTGGTGA  
6301 ACCAGGCCAG CCACGTTTCT GCGAAAACGC GGGAAAAAGT GGAAGCGGCG ATGGCGGAGC  
6361 TGAATTACAT TCCCAACCGC GTGGCACAAC AACTGGCGGG CAAACAGTCG TTGCTGATTG  
6421 GCGTTGCCAC CTCCAGTCTG GCCCTGCACG CGCCGTCGCA AATTGTCGCG GCGATTAAAT  
6481 CTCGCGCCGA TCAACTGGGT GCCAGCGTGG TGGTGTGAT GGTAGAACGA AGCGGCGTCG  
6541 AAGCCTGTAA AGC

FIGURE 22D

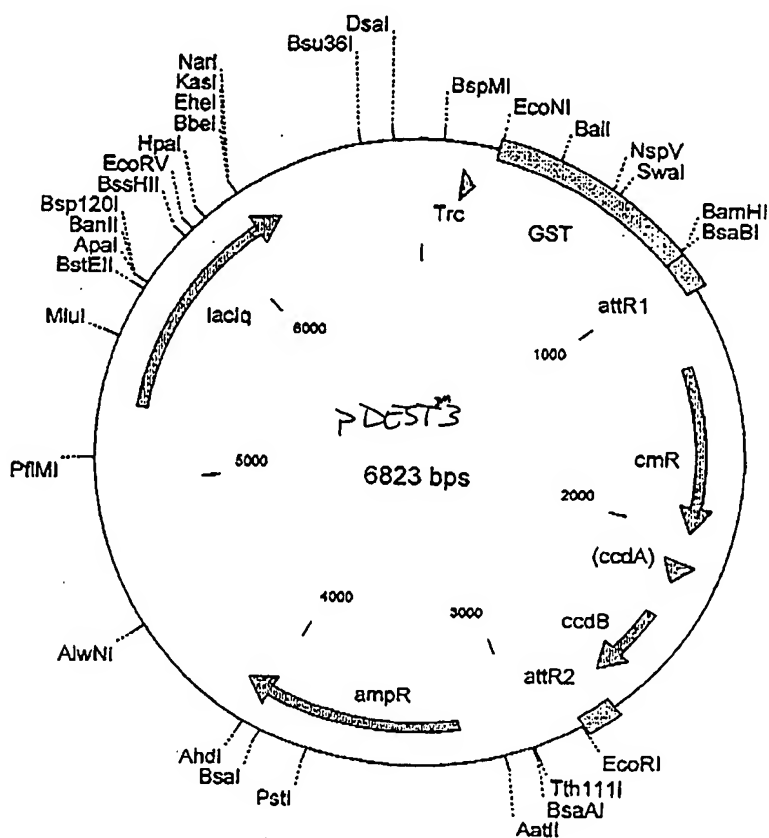
40/240

Figure 23A: pDEST3

## GST fusions in E. coli

154 cgg ttc tgg caa ata ttc tga aat gag ctg <sup>-35</sup> ttg aca att aat cat cgg ctc  
 gcc aag acc gtt tat aag act tta ctc gac <sup>-10</sup> aac tgt taa tta gta gcc gag  
 205 gta taa tgt gtg gaa ttg tga gcg gat aac aat ttc aca cag gaa aca gta  
 cat att aca cac ctt aac act cgc cta ttg tta aag tgt gtc ctt tgt cat  
 256 ttc atg tcc cct ata cta ggt tat tgg aaa att aag ggc ctt gtg caa ccc  
 aag tac agg gga tat gat cca ata acc ttt taa ttc ccg gaa cac gtt ggg

919 " GST → R G S R R A S V G S P S T S  
 ctg gtt ccg cgt gga tct cgt cgt gca tct gtt gga tcc cca tca aca agt  
 gac caa ggc gca cct aga gca gca cgt aga caa cct agg ggt agt tgt tca  
 970 ~~4 Y K K~~  
~~tgg ttc aac aac gct gaa cga gaa acg taa aat gat ata aat aat aat ata~~  
~~aac atg ttt ttt cga cct gct cct tgc att tta cta tat tta tag tta tat~~



41/240

## pDEST3 6823 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
150..200		Trc
1087..963		attR1
1337..1996		CmR
2116..2200		inactivated ccdA
2338..2643		ccdB
2684..2808		attR2
3231..4091		ampR
5295..6254		lacIq
1	ACGTTATCGA CTGCACGGTG CACCAATGCT TCTGGCGTCA GGCAGCCATC GGAAGCTGTG	
61	GTATGGCTGT GCAGGTCGTA AATCACTGCA TAATTCGTGT CGCTCAAGGC GCACTCCCGT	
121	TCTGGATAAT GTTTTTTGCG CCGACATCAT AACGGTTCGT GCAAATATTC TGAATGAGC	
181	TGTTGACAAT TAATCATCGG CTCGTATAAT GTGTGGAATT GTGAGCGGAT AACAAATTTCA	
241	CACAGGAAAC AGTATTCATG TCCCCTATAC TAGGTTATTG GAAAATTAAG GGCCTTGTGC	
301	AACCCACTCG ACTTCTTTTG GAATATCTTG AAGAAAAATA TGAAGAGCAT TTGTATGAGC	
361	GCGATGAAGG TGATAAATGG CGAAACAAAA AGTTTGAATT GGGTTTGGAG TTTCCCAATC	
421	TTCTTTATTA TATTGATGGT GATGTTAAAT TAACACAGTC TATGGCCATC ATACGTTATA	
481	TAGCTGACAA GCACAACATG TTGGGTGGTT GTCCAAAAGA GCGTGCAGAG ATTTCAATGC	
541	TTGAAGGAGC GGTTTTGGAT ATTAGATACG GTGTTTCGAG AATTGCATAT AGTAAAGACT	
601	TTGAAACTCT CAAAGTTGAT TTTCTTAGCA AGCTACCTGA AATGCTGAAA ATGTTCTGAAG	
661	ATCGTTTATG TCATAAAACA TATTTAAATG GTGATCATGT AACCCATCCT GACTTCATGT	
721	TGTATGACGC TCTTGATGTT GTTTTATACA TGGACCCAAT GTGCCTGGAT GCGTTCCCAA	
781	AATTAGTTTG TTTTAAAAAA CGTATTGAAG CTATCCCACA AATTGATAAG TACTTGAAAT	
841	CCAGCAAGTA TATAGCATGG CCTTTGCAGG GCTGGCAAGC CACGTTTGGT GGTGGCGACC	
901	ATCCTCCAAA ATCGGATCTG GTTCCGCGTG GATCTCGTCG TGCATCTGTT GGATCCCAT	
961	CAACAAGTTT GTACAAAAAA GCTGAACGAG AAACGTAAAA TGATATAAAT ATCAATATAT	
1021	TAAATTAGAT TTTGCATAAA AAACAGACTA CATAATACTG TAAAACACAA CATATCCAGT	
1081	CACTATGGCG GCCGCTAAGT TGGCAGCATC ACCCGACGCA CTTTGGCCCG AATAAATACC	
1141	TGTGACGGAA GATCACTTCG CAGAATAAAT AAATCCTGGT GTCCCTGTTG ATACCGGGAA	
1201	GCCCTGGGCC AACTTTTGGC GAAAATGAGA CGTTGATCGG CACGTAAGAG GTTCCAACCT	
1261	TCACCATAAT GAAATAAGAT CACTACCGGG CGTATTTTTT GAGTTATCGA GATTTTCAGG	
1321	AGCTAAGGAA GCTAAAATGG AGAAAAAAT CACTGGATAT ACCACCGTTG ATATATCCCA	
1381	ATGGCATCGT AAAGAACATT TTGAGGCATT TCAGTCAGTT GCTCAATGTA CCTATAACCA	
1441	GACCGTTCAG CTGGATATTA CGGCCTTTTT AAAGACCGTA AAGAAAAATA AGCACAAGTT	
1501	TTATCCGGCC TTTATTCACT TTCTTGCCCG CCTGATGAAT GCTCATCCGG AATTCCGTAT	
1561	GGCAATGAAA GACGGTGAGC TGGTGATATG GGATAGTGTT CACCCTTGTT ACACCGTTTT	
1621	CCATGAGCAA ACTGAAACGT TTTCATCGCT CTGGAGTGAA TACCACGACG ATTTCCGGCA	
1681	GTTTCTACAC ATATATTCGC AAGATGTGGC GTGTTACGGT GAAAACCTGG CCTATTTCCC	
1741	TAAAGGGTTT ATTGAGAATA TGTTTTTCGT CTCAGCCAAT CCCTGGGTGA GTTTCACCA	
1801	TTTTGATTTA AACGTGGCCA ATATGGACAA CTTCTTCGCC CCCGTTTTCA CCATGGGCAA	
1861	ATATTATACG CAAGGCGACA AGGTGCTGAT GCCGCTGGCG ATTCAGGTTC ATCATGCCGT	
1921	CTGTGATGGC TTCCATGTCG GCAGAATGCT TAATGAATTA CAACAGTACT GCGATGAGTG	
1981	GCAGGGCGGG GCGTAAAGAT CTGGATCCGG CTTACTAAAA GCCAGATAAC AGTATGCGTA	
2041	TTTGCGCGCT GATTTTTGCG GTATAAGAAT ATATACTGAT ATGTATACCC GAAGTATGTC	
2101	AAAAAGAGGT GTGCTATGAA GCAGCGTATT ACAGTGACAG TTGACACGGA CAGCTATCAG	
2161	TTGCTCAAGG CATATATGAT GTCAATATCT CCGTCTGGT AAGCACAAAC ATGCAGAATG	
2221	AAGCCCGTCG TCTGCGTGCC GAACGCTGGA AAGCGGAAAA TCAGGAAGGG ATGGCTGAGG	
2281	TCGCCCGGTT TATTGAAATG AACGGCTCTT TTGCTGACGA GAACAGGGAC TGGTGAAATG	
2341	CAGTTTAAGG TTTACACCTA TAAAAGAGAG AGCCGTTATC GTCTGTTTGT GGATGTACAG	
2401	AGTGATATTA TTGACACGCG CGGGCGACGG ATGGTGATCC CCCTGGCCAG TGCACGCTG	
2461	CTGTCTAGATA AAGTCTCCCG TGAACCTTAC CCGTGTTGTC ATATCGGGGA TGAAGCTGG	
2521	CGCATGATGA CCACCGATAT GGCCAGTGTG CCGGTCTCCG TTATCGGGGA AGAAGTGGCT	
2581	GATCTCAGCC ACCGCGAAAA TGACATCAAA AACGCCATTA ACCTGATGTT CTGGGGAATA	
2641	TAAATGTCAG GCTCCCTTAT ACACAGCCAG TCTGCAGGTC GACCATAGTG ACTGGATATG-	

FIGURE 23B

42/240

2701 TTGTGTTTTA CAGTATTATG TAGTCTGTTT TTTATGCAAA ATCTAATTTA ATATATTGAT  
2761 ATTTATATCA TTTTACGTTT CTCGTTCCAGC TTTCTTGTAAC AAAGTGGTTG ATGGGAATTC  
2821 ATCGTGACTG ACTGACGATC TGCCTCGCGC GTTTCGGTGA TGACGGTGAA AACCTCTGAC  
2881 ACATGCAGCT CCCGGAGACG GTCACAGCTT GTCTGTAAGC GGATGCCGGG AGCAGACAAG  
2941 CCCGTCAGGG CGCGTCAGCG GGTGTTGGCG GGTGTCGGGG CGCAGCCATG ACCCAGTCAC  
3001 GTAGCGATAG CGGAGTGTAT AATTCTTGAA GACGAAAGGG CCTCGTGATA CGCCTATTTT  
3061 TATAGGTTAA TGTCATGATA ATAATGGTTT CTTAGACGTC AGGTGGCACT TTTCCGGGAA  
3121 ATGTGCGCGG AACCCCTATT TGTATTATTT TCTAAATACA TTCAAATATG TATCCGCTCA  
3181 TGAGACAATA ACCCTGATAA ATGCTTCAAT AATATTGAAA AAGGAAGAGT ATGAGTATTC  
3241 AACATTTCCG TGTCGCCCTT ATTCCCTTTT TTGCGGCATT TTGCCTTCCT GTTTTGTGCT  
3301 ACCCAGAAAC GCTGGTGAAA GTAAAAGATG CTGAAGATCA GTTGGGTGCA CGAGTGGGTT  
3361 ACATCGAAGT GGATCTCAAC AGCGGTAAGA TCCTTGAGAG TTTTCGCCCC GAAGAACGTT  
3421 TTCCAATGAT GAGCACTTTT AAAGTCTGTC TATGTGGCGC GGTATTATCC CGTGTGACG  
3481 CCGGGCAAGA GCAACTCGGT CGCCGCATAC ACTATTCTCA GAATGACTTG GTTGAGTACT  
3541 CACCAGTCAC AGAAAAGCAT CTTACGGATG GCATGACAGT AAGAGAATTA TGCAGTGCTG  
3601 CCATAACCAT GAGTGATAAC ACTGCGGCCA ACTTACTTCT GACAACGATC GGAGGACCGA  
3661 AGGAGCTAAC CGCTTTTGTG CACAACATGG GGGATCATGT AACTCGCTT GATCGTTGGG  
3721 AACCGGAGCT GAATGAAGCC ATACCAAACG ACGAGCGTGA CACCACGATG CCTGCAGCAA  
3781 TGGCAACAAC GTTGCGCAAA CTATTAACATG GCGAACTACT TACTCTAGCT TCCCGGCAAC  
3841 AATTAATAGA CTGGATGGAG GCGGATAAAG TTGCAGGACC ACTTCTGCGC TCGGCCCTTC  
3901 CGGCTGGCTG GTTTATTGCT GATAAATCTG GAGCCGGTGA GCGTGGGTCT CGCGGTATCA  
3961 TTGCAGCACT GGGGCCAGAT GGTAAGCCCT CCCGTATCGT AGTTATCTAC ACAGCGGGGA  
4021 GTCAGGCAAC TATGGATGAA CGAAATAGAC AGATCGCTGA GATAGGTGCC TCAGTATTA  
4081 AGCATTGGTA ACTGTCAGAC CAAGTTTACT CATATATACT TTAGATTGAT TTA AAAACTTC  
4141 ATTTTAAATT TAAAAGGATC TAGGTGAAGA TCCTTTTGA TAATCTCATG ACCAAAATCC  
4201 CTTAACGTGA GTTTTCGTTT CACTGAGCGT CAGACCCCGT AGAAAAGATC AAAGGATCTT  
4261 CTTGAGATCC TTTTTCGTTG CGCGTAATCT GCTGCTTGCA AACAAAAAAA CCACCGCTAC  
4321 CAGCGGTGGT TTGTTTGCCG GATCAAGAGC TACCAACTCT TTTTCCGAAG GTAACGTGGT  
4381 TCAGCAGAGC GCAGATACCA AATACTGTCC TTCTAGTGTA GCCGTAGTTA GGCCACCACT  
4441 TCAAGAACTC TGTAGCACCG CCTACATACC TCGCTCTGCT AATCCTGTGA CCAGTGGCTG  
4501 CTGCCAGTGG CGATAAGTCG TGTCTTACCG GGTGGGACTC AAGACGATAG TTACCGGATA  
4561 AGGCGCAGCG GTCGGGCTGA ACGGGGGGTT CGTGACACACA GCCCAGCTTG GAGCGAACGA  
4621 CCTACACCGA ACTGAGATAC CTACAGCGTG AGCTATGAGA AAGCGCCACG CTTCCCGAAG  
4681 GGAGAAAGGC GGACAGGTAT CCGGTAAGCG GCAGGGTCGG AACAGGAGAG CGCACGAGGG  
4741 AGCTTCCAGG GGGAAACGCC TGGTATCTTT ATAGTCCTGT CGGGTTTCGC CACCTCTGAC  
4801 TTGAGCGTCG ATTTTGTGTA TGCTCGTCAG GGGGGCGGAG CCTATGGAAA AACGCCAGCA  
4861 ACGCGGCCTT TTTACGGTTC CTGGCCTTTT GCTGGCCTTT TGCTCACATG TTCTTCTCTG  
4921 CGTTATCCCC TGATTCTGTG GATAACCGTA TTACCGCCTT TGAGTGAGCT GATACCGCTC  
4981 GCCGCAGCCG AACGACCGAG CGCAGCGAGT CAGTGAGCGA GGAAGCGGAA GAGCGCCTGA  
5041 TGCGGTATTT TCTCCTTACG CATCTGTGCG GTATTTTACA CCGCATAAAT TCCGACACCA  
5101 TCGAATGGTG CAAAACCTTT CGCGGTATGG CATGATAGCG CCCGGAAGAG AGTCAATTCA  
5161 GGGTGGTGAA TGTGAAACCA GTAACGTTAT ACGATGTGCG AGAGTATGCC GGTGTCTCTT  
5221 ATCAGACCGT TTCCCGCGTG GTGAACCAGG CCAGCCACGT TTCTGCGAAA ACGCGGGAAA  
5281 AAGTGGAAGC GGCGATGGCG GAGCTGAATT ACATTCCCAA CCGCGTGGCA CAACAACTGG  
5341 CCGGCAAACA GTCGTTGCTG ATTGGCGTTG CCACCTCCAG TCTGGCCCTG CACGCGCCGT  
5401 CGCAAATTGT CGCGGCGATT AAATCTCGCG CCGATCAACT GGGTGCCAGC GTGGTGGTGT  
5461 CGATGGTAGA ACGAAGCGGC GTCGAAGCCT GTAAAGCGGC GGTGCACAAT CTCTCGCGC  
5521 AACGCGTCAG TGGGCTGATC ATTAACATATC CGCTGGATGA CCAGGATGCC ATTGCTGTGG  
5581 AAGCTGCCTG CACTAATGTT CCGGCGTTAT TTCTTGATGT CTCTGACCAG ACACCCATCA  
5641 ACAGTATTAT TTTCTCCCAT GAAGACGGTA CGCGACTGGG CGTGGAGCAT CTGGTCGCAT  
5701 TGGGTCACCA GCAAATCGCG CTGTTAGCGG GCCCATTAAG TTCTGTCTCG GCGCGTCTGC  
5761 GTCTGGCTGG CTGGCATAAA TATCTCACTC GCAATCAAAT TCAGCCGATA GCGGAACGGG  
5821 AAGGCGACTG GAGTGCCATG TCCGGTTTTC AACAAACCAT GCAAATGCTG AATGAGGGCA  
5881 TCGTTCCAC TGCGATGCTG GTTGCCAACG ATCAGATGGC GCTGGGCGCA ATGCGCGCCA  
5941 TTACCGAGTC CGGGCTGCGC GTTGGTGCGG ATATCTCGGT AGTGGGATAC GACGATACCG  
6001 AAGACAGCTC ATGTTATATC CCGCCGTTAA CCACCATCAA ACAGGATTTT CGCCTGCTGG  
6061 GGCAAACCGC CGTGGACCGC TTGCTGCAAC TCTCTCAGGG CCAGGCGGTG AAGGGCAATC  
6121 AGCTGTTGCC CGTCTCACTG GTGAAAAGAA AAACCACCCT GGCGCCCAAT ACGCAAACCG-

FIGURE 23C



43/240

6181 CCTCTCCCCG CGCGTTGGCC GATTCATTAA TGCAGCTGGC ACGACAGGTT TCCCGACTGG  
6241 AAAGCGGGCA GTGAGCGCAA CGCAATTAAT GTGAGTTAGC TCACTCATTG GGCACCCCAG  
6301 GCTTTACACT TTATGCTTCC GGCTCGTATG TTGTGTGGAA TTGTGAGCGG ATAACAATTT  
6361 CACACAGGAA ACAGCTATGA CCATGATTAC GGATTCACGT GCCGTCGTTT TACAACGTCG  
6421 TGA CTGGGAA AACCCTGGCG TTACCCAACT TAATCGCCTT GCAGCACATC CCCCTTTTCGC  
6481 CAGCTGGCGT AATAGCGAAG AGGCCCGCAC CGATCGCCCT TCCCAACAGT TGCGCAGCCT  
6541 GAATGGCGAA TGGCGCTTTG CCTGGTTTCC GGCACCAGAA GCGGTGCCGG AAAGCTGGCT  
6601 GGAGTGCGAT CTTCTGAGG CCGATACTGT CGTCGTCCCC TCAAACCTGGC AGATGCACGG  
6661 TTACGATGCG CCCATCTACA CCAACGTAAC CTATCCCATT ACGGTCAATC CGCCGTTTGT  
6721 TCCCACGGAG AATCCGACGG GTTGTTACTC GCTCACATT AATGTTGATG AAAGCTGGCT  
6781 ACAGGAAGGC CAGACGCGAA TTATTTTGA TGGCGTTGGA ATT

FIGURE 23D



45/240

## pDEST4 6964 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
964..1003		Trc
1577..1453		attR1
1827..2486		CmR
2606..2690		inactivated ccdA
2828..3133		ccdB
3174..3298		attR2
3872..4777		ampR
5378..5538		ori
5778..6215		flori (f1 intergenic region)
6587..704		lacIq

1	CTATCCGCTG	GATGACCAGG	ATGCCATTGC	TGTGGAAGCT	GCCTGCACTA	ATGTTCCGGC
61	GTTATTTCTT	GATGTCTCTG	ACCAGACACC	CATCAACAGT	ATTATTTTCT	CCCATGAAGA
121	CGGTACGCGA	CTGGGCGTGG	AGCATCTGGT	CGCATTGGGT	CACCAGCAAA	TCGCGCTGTT
181	AGCGGGCCCA	TTAAGTTCTG	TCTCGGCGCG	TCTGCGTCTG	GCTGGCTGGC	ATAAATATCT
241	CACTCGCAAT	CAAATTCAGC	CGATAGCGGA	ACGGGAAGGC	GACTGGAGTG	CCATGTCCGG
301	TTTTCAACAA	ACCATGCAAA	TGCTGAATGA	GGGCATCGTT	CCCACTGCGA	TGCTGGTTGC
361	CAACGATCAG	ATGGGCGTGG	GCGCAATGCG	CGCCATTACC	GAGTCCGGGC	TGCGCGTTGG
421	TGCGGATATC	TCGGTAGTGG	GATACGACGA	TACCGAAGAC	AGCTCATGTT	ATATCCCGCC
481	GTCAACCACC	ATCAAACAGG	ATTTTCGCCT	GCTGGGGCAA	ACCAGCGTGG	ACCGCTTGCT
541	GCAACTCTCT	CAGGGCCAGG	CGGTGAAGGG	CAATCAGCTG	TTGCCCGTCT	CACCTGGTGAA
601	AAGAAAAACC	ACCCTGGCAC	CCAATACGCA	AACCGCCTCT	CCCCGCGCGT	TGGCCGATTC
661	ATTAATGCAG	CTGGCACGAC	AGGTTTCCCG	ACTGGAAGC	GGGCAGTGAG	CGCAACGCAA
721	TTAATGTGAG	TTAGCGCGAA	TTGATCTGGT	TTGACAGCTT	ATCATCGACT	GCACGGTGCA
781	CCAATGCTTC	TGGCGTCAGG	CAGCCATCGG	AAGCTGTGGT	ATGGCTGTGC	AGGTCGTAAA
841	TCACTGCATA	ATTCGTGTGC	CTCAAGGCGC	ACTCCCGTTC	TGGATAATGT	TTTTTGCGCC
901	GACATCATAA	CGGTTCTGGC	AAATATTCTG	AAATGAGCTG	TTGACAATTA	ATCATCCGGT
961	CCGTATAATC	TGTGGAATTG	TGAGCGGATA	ACAATTTTAC	ACAGGAAACA	GACCATGGGT
1021	CATCATCATC	ATCATCACGA	TTACGATATC	CCAACGACCG	AAAACCTGTA	TTTTCAGGGC
1081	GCCCATATGA	GCGATAAAAT	TATTCACCTG	ACTGACGACA	GTTTTGACAC	GGATGTACTC
1141	AAAGCGGACG	GGGCGATCCT	CGTCGATTTC	TGGGCAGAGT	GGTGCGGTCC	GTGCAAAATG
1201	ATCGCCCCGA	TTCTGGATGA	AATCGCTGAC	GAATATCAGG	GCAAACTGAC	CGTTGCAAAA
1261	CTGAACATCG	ATCAAAAACC	TGGCACTGCG	CCGAAATATG	GCATCCGTGG	TATCCCGACT
1321	CTGCTGCTGT	TCAAAAACGG	TGAAGTGGCG	GCAACCAAAG	TGGGTGCACT	GTCTAAAGGT
1381	CAGTTGAAAG	AGTTCCCTCGA	CGCTAACCTG	GCCGGTTCTG	GTTCTGGTGA	TGACGATGAC
1441	AAGGTACCCA	TCACAAGTTT	GTACAAAAAA	GCTGAACGAG	AAACGTAAAA	TGATATAAAT
1501	ATCAATATAT	TAAATTAGAT	TTTGCAATAA	AAACAGACTA	CATAATACTG	TAAAACACAA
1561	CATATCCAGT	CACTATGGCG	GCCGCTAAGT	TGGCAGCATC	ACCCGACGCA	CTTTGCGCCG
1621	AATAAATACC	TGTGACGGAA	GATCACTTCG	CAGAATAAAT	AAATCCTGGT	GTCCTGTGTT
1681	ATACCGGGAA	GCCCTGGGCC	AACTTTTGGC	GAAAATGAGA	CGTTGATCGG	CACGTAAGAG
1741	GTTCCAACCT	TCACCATAAT	GAAATAAGAT	CACTACCGGG	CGTATTTTTT	GAGTTATCGA
1801	GATTTTCAGG	AGCTAAGGAA	GCTAAAATGG	AGAAAAAAAT	CACTGGATAT	ACCACCGTTG
1861	ATATATCCCA	ATGGCATCGT	AAAGAACATT	TTGAGGCATT	TCAGTCAGTT	GCTCAATGTA
1921	CCTATAACCA	GACCGTTTCA	CTGGATATTA	CGGCCTTTTT	AAAGACCGTA	AAGAAAAATA
1981	AGCAACAAGT	TTATCCGGCC	TTTATTCACA	TTCTTGCCCG	CCTGATGAAT	GCTCATCCGG
2041	AATTCCGTAT	GGCAATGAAA	GACGGTGAGC	TGGTGATATG	GGATAGTGT	CACCCTTGTT
2101	ACACCGTTTT	CCATGAGCAA	ACTGAAACGT	TTTCATCGCT	CTGGAGTGAA	TACCACGACG
2161	ATTTCCGGCA	GTTTCTACAC	ATATATTTCG	AAGATGTGGC	GTGTTACGGT	GAAAACCTGG
2221	CCTATTTCCC	TAAAGGGTTT	ATTGAGAATA	TGTTTTTCGT	CTCAGCCAAT	CCCTGGGTGA
2281	GTTTCACCAG	TTTTGATTTA	AACGTGGCCA	ATATGGACAA	CTTCTTCGCG	CCCGTTTCA
2341	CCATGGGCAA	ATATTATACG	CAAGGCGACA	AGGTGCTGAT	GCCGCTGGCG	ATTGAGGTTT
2401	ATCATGCCGT	CTGTGATGGC	TTCCATGTCC	GCAGAATGCT	TAATGAATTA	CAACAGTACT
2461	GCGATGAGTG	GCAGGGCGGG	GCGTAAACGC	GTGGATCCGG	CTTACTAAAA	GCCAGATAAC
2521	AGTATGCGTA	TTTGCGCGCT	GATTTTTGCG	GTATAAGAAT	ATATACTGAT	ATGTATACCC-

FIGURE 24B

46/240

2581 GAAGTATGTC AAAAAGAGGT GTGCTATGAA GCAGCGTATT ACAGTGACAG TTGACAGCGA  
2641 CAGCTATCAG TTGCTCAAGG CATATATGAT GTCAATATCT CCGGTCTGGT AAGCACAACC  
2701 ATGCAGAATG AAGCCCGTCG TCTGCGTGCC GAACGCTGGA AAGCGGAAAA TCAGGAAGGG  
2761 ATGGCTGAGG TCGCCCGGTT TATTGAAATG AACGGCTCTT TTGCTGACGA GAACAGGGAC  
2821 TGGTGAAATG CAGTTTAAGG TTTACACCTA TAAAGAGAG AGCCGTTATC GTCTGTTTGT  
2881 GGATGTACAG AGTGATATTA TTGACACGCC CGGGCGACGG ATGGTGATCC CCCTGGCCAG  
2941 TGCACGCTCG CTGTCAGATA AAGTCTCCCG TGAACTTTAC CCGGTGGTGC ATATCGGGGA  
3001 TGAAAGCTGG CGCATGATGA CCACCGATAT GGCCAGTGTG CCGGTCTCCG TTATCGGGGA  
3061 AGAAGTGGCT GATCTCAGCC ACCGCGAAAA TGACATCAAA AACGCCATTA ACCTGATGTT  
3121 CTGGGGAATA TAAATGTCAG GCTCCCTTAT ACACAGCCAG TCTGCAGGTC GACCATAGTG  
3181 ATGGGATATG TTGTGTTTTA CAGTATTATG TAGTCTGTTT TTTATGCAAA ATCTAATTTA  
3241 ATATATTGAT ATTTATATCA TTTTACGTTT CTCGTTGACG TTTCTTGATC AAAGTGGTGA  
3301 TGGGGATCCT CTAGAGTCGA CCTGCAGTAA TCGTACAGGG TAGTACAAAT AAAAAAGGCA  
3361 CGTCAGATGA CGTGCCCTTTT TTCTTGAGAG CAGTAAGCTT GGCTGTTTTG GCGGATGAGA  
3421 GAAGATTTTC AGCCTGATAC AGATTAAATC AGAACGCAGA AGCGGTCTGA TAAACAGAA  
3481 TTTGCCCTGGC GGCAGTAGCG CGGTGGTCCC ACCTGACCCC ATGCCGAAC TCAAGTGAA  
3541 ACGCCGTAGC GCCGATGGTA GTGTGGGGTC TCCCCATGCG AGAGTAGGGA ACTGCCAGGC  
3601 ATCAAATAAA ACGAAAGGCT CAGTCGAAAG ACTGGGCTTT TCGTTTTATC TGTGTTTGT  
3661 CGGTGAACGC TCTCTGAGT AGGACAAATC CGCCGGGAGC GGATTTGAAC GTTGCGAAGC  
3721 AACGGCCCGG AGGGTGGCGG GCAGGACGCC CGCCATAAAC TGCCAGGCAT CAAATTAAGC  
3781 AGAAGGCCAT CCTGACGGAT GGCCTTTTTG CGTTTCTACA AACTCTTTTT GTTTATTTTT  
3841 CTAATACAT TCAAATATGT ATCCGCTCAT GAGACAATAA CCCTGATAAA TGCTTCAATA  
3901 ATATTGAAAA AGGAAGAGTA TGAGTATTCA ACATTTCCGT GTCGCCCTTA TTCCCTTTTT  
3961 TGCGGCATTT TGCCTTCCTG TTTTGGCTCA CCCAGAAACG CTGGTGAAGC TAAAGATGC  
4021 TGAAGATCAG TTGGGTGCAC GAGTGGGTTA CATCGAACTG GATCTCAACA GCGGTAAGAT  
4081 CCTTGAGAGT TTTGCCCCG AAGAACGTTT TCCAATGATG AGCACTTTTA AAGTTCTGCT  
4141 ATGTGGCGCG GTATTATCCC GTGTTGACGC CGGGCAAGAG CAACTCGGTC GCCGCATACA  
4201 CTATTCTCAG AATGACTTGG TTGAGTACTC ACCAGTCACA GAAAAGCATC TTACGGATGG  
4261 CTAGACAGTA AGAGAATTAT GCAGTGCTGC CATAACCATG AGTGATAACA CTGCGGCCAA  
4321 CTTACTTCTG ACAACGATCG GAGGACCGAA GGAGCTAACC GCTTTTTTGC ACAACATGGG  
4381 GGATCATGTA ACTCGCCTTG ATCGTTGGGA ACCGGAGCTG AATGAAGCCA TACCAAACGA  
4441 CGAGCGTGAC ACCACGATGC CTACAGCAAT GGCAACAACG TTGCGCAAA TATTAAGTGG  
4501 CGAACTACTT ACTCTAGCTT CCCGGCAACA ATTAATAGAC TGGATGGAGG CGGATAAAGT  
4561 TGCAAGACCA CTTCTGCGCT CGGCCCTTCC GGCTGGCTGG TTTATGCTG ATAAATCTGG  
4621 AGCCGGTGAG CGTGGGTCTC GCGGTATCAT TGCAAGCACTG GGGCCAGATG GTAAGCCCTC  
4681 CCGTATCGTA GTTATCTACA CGACGGGGAG TCAGGCAACT ATGGATGAAC GAAATAGACA  
4741 GATCGCTGAG ATAGGTGCCT CACTGATTAA GCATTGGTAA CTGTCAGACC AAGTTTACTC  
4801 ATATATACTT TAGATTGATT TAAAACCTCA TTTTAAATTT AAAAGGATCT AGGTGAAGAT  
4861 CCTTTTGTAT AATCTCATGA CCAAAATCCC TTAACGTGAG TTTTCGTTCC ACTGAGCGTC  
4921 AGACCCCGTA GAAAAGATCA AAGGATCTTC TTGAGATCCT TTTTCTGTC GCGTAATCTG  
4981 CTGCTTGCAA ACAAAAAAAC CACCGCTACC AGCGGTGGTT TGTTTGCCCG ATCAAGAGCT  
5041 ACCAACTCTT TTTCCGAAGG TAAGTGGCTT CAGCAGAGCG CAGATACCAA ATACTGTCTT  
5101 TCTAGTGTAG CCGTAGTTAG GCCACCACTT CAAGAACTCT GTAGCACCAG CTACATACCT  
5161 CGCTCTGCTA ATCCTGTTAC CAGTGGCTGC TGCCAGTGGC GATAAGTCGT GTCTTACCGG  
5221 GTTGGACTCA AGACGATAGT TACCGGATAA GGCGCAGCGG TCGGGCTGAA CGGGGGGTTT  
5281 GTGCACACAG CCCAGCTTGG AGCGAACGAC CTACACCGAA CTGAGATACC TACAGCGTGA  
5341 GCTATGAGAA AGCGCCACGC TTCCCGAAGG GAGAAAGGCG GACAGGTATC CGGTAAGCGG  
5401 CAGGGTCCGA ACAGGAGAGC GCACGAGGGA GCTTCCAGGG GGAAACGCCT GGTATCTTTA  
5461 TAGTCCTGTC GGGTTTCGCC ACCTCTGACT TGAGCGTCGA TTTTGTGAT GCTCGTCAGG  
5521 GGGGCGGAGC CTATGGAAAA ACGCCAGCAA CGCGGCCTTT TTACGGTTCC TGGCCTTTTG  
5581 CTGGCCTTTT GCTCACATGT TCTTCTGTC GTTATCCCTT GATTCTGTGG ATAACCGTAT  
5641 TACCGCCTTT GAGTGAGCTG ATACCGCTCG CCGCAGCCGA ACGACCGAGC GCAGCGAGTC  
5701 AGTGAGCGAG GAAGCGGAAG AGCGCCTGAT GCGGTATTTT CTCCTTACGC ATCTGTGCGG  
5761 TATTTACAC CGCATAATTT TGTTAAATTT CGCGTTAAAT TTTTGTAAAA TCAGTCTATT  
5821 TTTTAACCAA TAGGCCGAAA TCGGCAAAAT CCCTTATAAA TCAAAAGAAT AGACCGAGAT  
5881 AGGGTTGAGT GTTGTTCAG TTTGGAACAA GAGTCCACTA TTAAAGAACG TGGACTCCAA  
5941 CGTCAAAGGG CGAAAAACCG TCTATCAGGG CGATGGCCCA CTAGGTGAAC CATCACCTTA  
6001 ATCAAGTTTT TTGGGGTCGA GGTGCCGTAA AGCACTAAAT CGGAACCTTA AAGGGAGCCC-

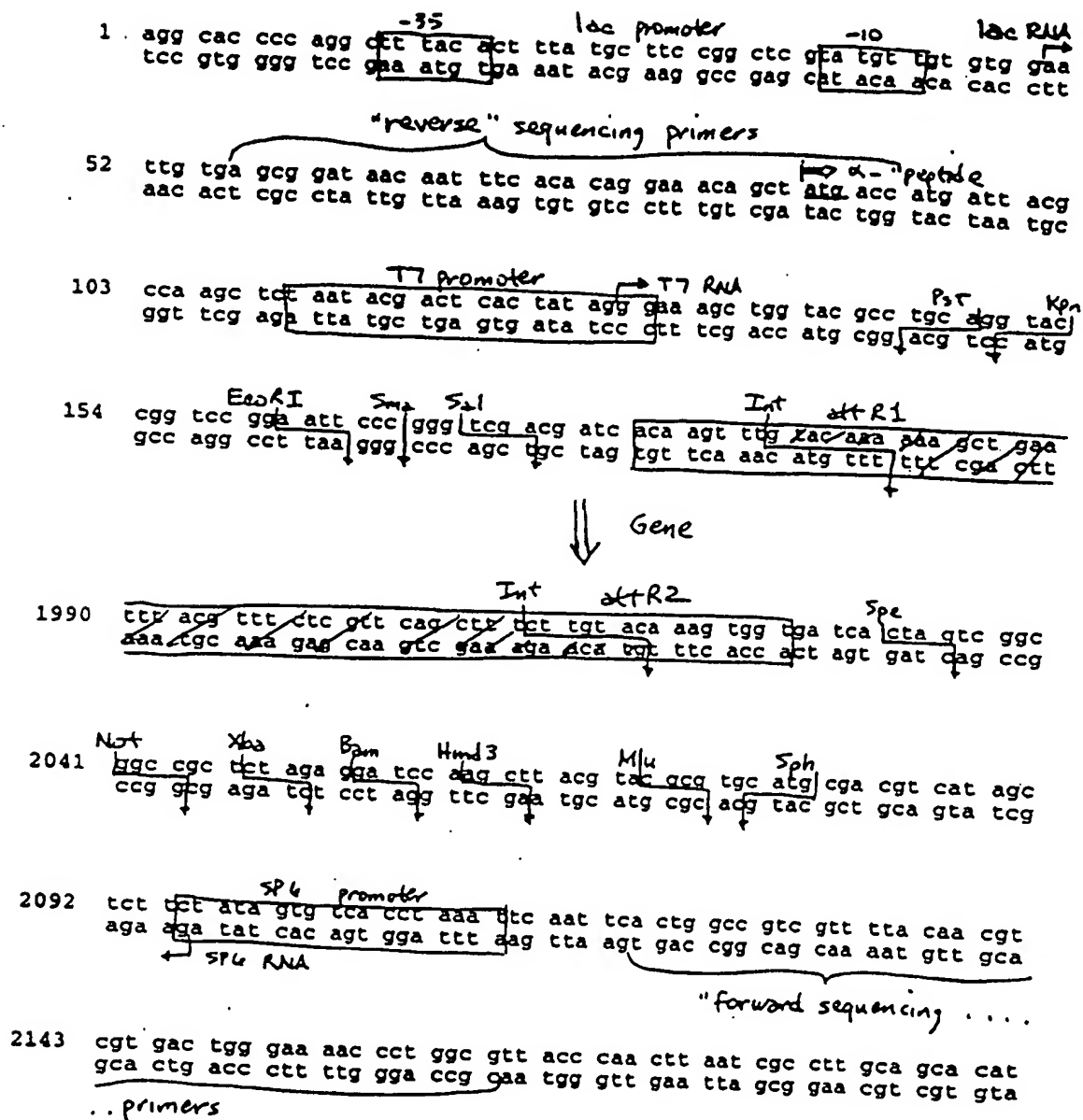
FIGURE 24C

6061 CCGATTTAGA GCTTGACGGG GAAAGCCGGC GAACGTGGCG AGAAAGGAAG GGAAGAAAGC  
6121 GAAAGGAGCG GCGGCTAGGG CGCTGGCAAG TGTAGCGGTC ACGCTGCGCG TAACCACCAC  
6181 ACCCGCCGCG CTTAATGCGC CGCTACAGGG CGCGTCCATT CGCCATTCAG GCTGCTATGG  
6241 TGCACTCTCA GTACAATCTG CTCTGATGCC GCATAGTTAA GCCAGTATAC ACTCCGCTAT  
6301 CGCTACGTGA CTGGGTCATG GCTGCGCCCC GACACCCGCC AACACCCGCT GACGCGCCCT  
6361 GACGGGCTTG TCTGCTCCCG GCATCCGCTT ACAGACAAGC TGTGACCGTC TCCGGGAGCT  
6421 GCATGTGTCA GAGGTTTTCA CCGTCATCAC CGAAACGCGC GAGGCAGCAG ATCAATTCGC  
6481 GCGCGAAGGC GAAGCGGCAT GCATTTACGT TGACACCATC GAATGGTGCA AAACCTTTCC  
6541 CGGTATGGCA TGATAGCGCC CGGAAGAGAG TCAATTCAGG GTGGTGAATG TGAAACCACT  
6601 AACGTTATAC GATGTCGCAG AGTATGCCGG TGTCTCTTAT CAGACCGTTT CCCGCGTGGT  
6661 GAACCAGGCC AGCCACGTTT CTGCGAAAAC GCGGGAAAAA GTGGAAGCGG CGATGGCGGA  
6721 GCTGAATTAC ATTCCCAACC GCGTGGCACA ACAACTGGCG GGCAAACAGT CGTTGCTGAT  
6781 TGGCGTTGCC ACCTCCAGTC TGGCCCTGCA CGCGCCGTCG CAAATTGTGCG CGGCGATTAA  
6841 ATCTCGCGCC GATCAACTGG GTGCCAGCGT GGTGGTGTGCG ATGGTAGAAC GAAGCGGCGT  
6901 CGAAGCCTGT AAAGCGGCGG TGCACAATCT TCTCGCGCAA CGCGTCAGTN GGGCTGATCA  
6961 TTAA

48/240

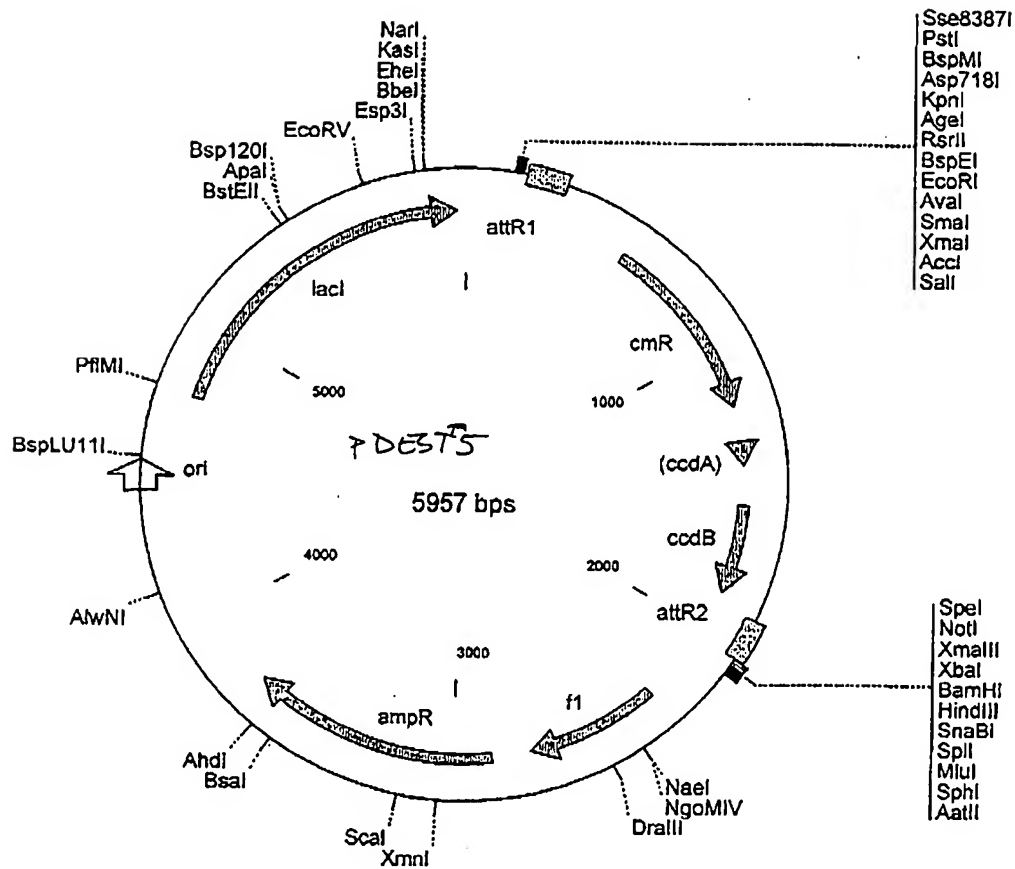
Figure 25A

pDEST5

pSPORT '+' (for sequencing, probes,  
phagemid)

49/260

Figure 25B  $\gamma$ DEST5 (cont'd)



50/240

## pDEST5 5957 bp

<u>Location (Base Nos.)</u>			<u>Gene Encoded</u>			
		305..181			attR1	
		555..1214			CmR	
		1334..1418			inactivated ccdA	
		1556..1861			ccdB	
		1902..2026			attR2	
		2278..2733			f1 (f1 intergenic region)	
		2865..3722			ampR	
		5378..5538			ori	
		4756..5922			lacI	
1	AGGCACCCCA	GGCTTTACAC	TTTATGCTTC	CGGCTCGTAT	GTTGTGTGGA	ATTGTGAGCG
61	GATAACAATT	TCACACAGGA	AACAGCTATG	ACCATGATTA	CGCCAAGCTC	TAATACGACT
121	CACATAGGG	AAAGCTGGTA	CGCCTGCAGG	TACCGGTCCG	GAATTCCCGG	GTGACGATC
181	ACAAGTTTGT	ACAAAAAAGC	TGAACGAGAA	ACGTAAAATG	ATATAAATAT	CAATATATTA
241	AATTAGATTT	TGCATAAAAA	ACAGACTACA	TAATACTGTA	AAACACAACA	TATCCAGTCA
301	CTATGGCGGC	CGCTAAGTTG	GCAGCATCAC	CCGACGCACT	TTGCGCCGAA	TAAATACCTG
361	TGACGGAAGA	TCACTTCGCA	GAATAAATAA	ATCCTGGTGT	CCCTGTTGAT	ACCGGGAAGC
421	CCTGGGCCAA	CTTTTGGCGA	AAATGAGACG	TTGATCGGCA	CGTAAGAGGT	TCCAACCTTC
481	ACCATAATGA	AATAAGATCA	CTACCGGGCG	TATTTTTTGA	GTTATCGAGA	TTTTCAGGAG
541	CTAAGGAAGC	TAAAATGGAG	AAAAAAATCA	CTGGATATAC	CACCGTTGAT	ATATCCCAAT
601	GGCATCGTAA	AGAACATTTT	GAGGCATTTT	AGTCAGTTGC	TCAATGTACC	TATAACCAGA
661	CCGTTTCAGCT	GGATATTACG	GCCTTTTTAA	AGACCGTAAA	GAAAAATAAG	CACAAGTTTT
721	ATCCGGCCTT	TATTCACATT	CTTGCCCGCC	TGATGAATGC	TCATCCGGAA	TCCGATATGG
781	CAATGAAAGA	CGGTGAGCTG	GTGATATGGG	ATAGTGTTC	CCCTTGTTAC	ACCGTTTTCC
841	ATGAGCAAAC	TGAAACGTTT	TCATCGCTCT	GGAGTGAATA	CCACGACGAT	TTCCGGCAGT
901	TTCTACACAT	ATATTTCGCA	GATGTGGCGT	GTTACGGTGA	AAACCTGGCC	TATTTCCCTA
961	AAGGGTTTAT	TGAGAATATG	TTTTTCGTCT	CAGCCAATCC	CTGGGTGAGT	TTCACCAGTT
1021	TTGATTTTAA	CGTGGCCAAT	ATGGACAAC	TCTTCGCCCC	CGTTTTTACC	ATGGGCAAA
1081	ATTATACGCA	AGGCGACAAG	GTGCTGATGC	CGCTGGCGAT	TCAGGTTTAT	CATGCCGTCT
1141	GTGATGGCTT	CCATGTCGGC	AGAATGCTTA	ATGAATTACA	ACAGTACTGC	GATGAGTGGC
1201	AGGGCGGGGC	GTAAACGCGT	GGATCCGGCT	TACTAAAAGC	CAGATAACAG	TATGCGTATT
1261	TGCGCGCTGA	TTTTTTCGGT	ATAAGAATAT	ATACTGATAT	GTATACCCGA	AGTATGTCAA
1321	AAAGAGGTGT	GCTATGAAGC	AGCGTATTAC	AGTGACAGTT	GACAGCGACA	GCTATCAGTT
1381	GCTCAAGGCA	TATATGATGT	CAATATCTCC	GGTCTGGTAA	GCACAACCAT	GCAGAATGAA
1441	GCCCGTCGTC	TGCGTGCCGA	ACGCTGGAAA	GCGGAAAATC	AGGAAGGGAT	GGCTGAGGTC
1501	GCCCGGTTTA	TGAAATGAA	CGGCTCTTTT	GCTGACGAGA	ACAGGGACTG	GTGAAATGCA
1561	GTTTAAGGTT	TACACCTATA	AAAGAGAGAG	CCGTTATCGT	CTGTTTGTGG	ATGTACAGAG
1621	TGATATTATT	GACACGCCCG	GGCGACGGAT	GGTGATCCCC	CTGGCCAGTG	CACGTCTGCT
1681	GTCAGATAAA	GTCTCCCGTG	AACTTTACCC	GGTGGTGCAT	ATCGGGGATG	AAAGCTGGCG
1741	CATGATGACC	ACCGATATGG	CCAGTGTGCC	GGTCTCCGTT	ATCGGGGAAG	AAGTGGCTGA
1801	TCTCAGCCAC	CGCGAAAATG	ACATCAAAAA	CGCCATTAA	CTGATGTTCT	GGGGAATATA
1861	AATGTCAGGC	TCCCTTATAC	ACAGCCAGTC	TGCAGGTCGA	CCATAGTGAC	TGGATATGTT
1921	GTGTTTAC	GTATTATGTA	GTCTGTTTTT	TATGCAAAAT	CTAATTTAAT	ATATTGATAT
1981	TTATATCATT	TTACGTTTCT	CGTTTCAGCTT	TCTTGTACAA	AGTGGTGATC	ACTAGTCGGC
2041	GGCCGCTCTA	GAGGATCCAA	GCTTACGTAC	GCGTGCATGC	GACGTCATAG	CTCTTCTATA
2101	GTGTCACCTA	AATTCAATT	ACTGGCCGTC	GTTTTTACAAC	GTCGTGACTG	GGAAAACCC
2161	GGCGTTTACC	AACTTAATCG	CCTTGCAGCA	CATCCCCCTT	TCGCCAGCTG	GCCTAATAGC
2221	GAAGAGGCCC	GCACCGATCG	CCCTTCCCAA	CAGTTGCGCA	GCCTGAATGG	CGAATGGACG
2281	CGCCCTGTAG	CGGCGCATT	AGCGCGGCGG	GTGTGGTGGT	TACGCGCAGC	GTGACCGCTA
2341	CAC TTGCCAG	CGCCCTAGCG	CCCCTCCTT	TCGCTTTCTT	CCCTTCCTTT	CTCGCCACGT
2401	TCGCCGGCTT	TCCCGTCAA	GCTCTAAATC	GGGGGCTCCC	TTTAGGGTTT	CGATTTAGTG
2461	CTTTACGGCA	CCTCGACCCC	AAAAAACTTG	ATTAGGGTGA	TGGTTCACGT	AGTGGGCCAT
2521	CGCCCTGATA	GACGGTTTTT	CGCCCTTTGA	CGTTGGAGTC	CACGTTCTTT	AATAGTGGAC
2581	TCTTGTTCCA	AACTGGAACA	ACACTCAACC	CTATCTCGGT	CTATCTTTTT	GATTTATAAG

FIGURE 25C



51/240

2641 GGATTTTGCC GATTTTCGGCC TATTGGTTAA AAAATGAGCT GATTTAACAA AAATTTAACG  
2701 CGAATTTTAA CAAAATATTA ACGTTTACAA TTTCAGGTGG CACTTTTCGG GGAAATGTGC  
2761 GCGGAACCCC TATTTGTTTA TTTTCTAAA TACATTCAA TATGTATCCG CTCATGAGAC  
2821 AATAACCCCTG ATAAATGCTT CAATAATATT GAAAAAGGAA GAGTATGAGT ATTCAACATT  
2881 TCCGTGTCGC CCTTATTCCC TTTTTCGCGG CATTITGCTT TCCTGTTTTT GCTCACCAG  
2941 AAACGCTGGT GAAAGTAAAA GATGCTGAAG ATCAGTTGGG TGCACGAGTG GGTACATCG  
3001 AACTGGATCT CAACAGCGGT AAGATCCTTG AGAGTTTTCG CCCCAGAGAA CGTTTCCAA  
3061 TGATGAGCAC TTTTAAAGTT CTGCTATGTG GCGCGGTATT ATCCCGTATT GACGCCGGG  
3121 AAGAGCAACT CGGTCGCCGC ATACACTATT CTCAGAATGA CTTGGTTGAG TACTCACCAG  
3181 TCACAGAAAA GCATCTTACG GATGGCATGA CAGTAAGAGA ATTATGCAGT GCTGCCATAA  
3241 CCATGAGTGA TAACACTGCG GCCAACTTAC TTCTGACAAC GATCGGAGGA CCGAAGGAGC  
3301 TAACCGCTTT TTTGCACAAC ATGGGGGATC ATGTAACCTG CCTTGATCGT TGGGAACCGG  
3361 AGCTGAATGA AGCCATACCA AACGACGAGC GTGACACCAC GATGCCTGTA GCAATGGCAA  
3421 CAACGTTGCG CAAACTATTA ACTGGCGAAC TACTTACTCT AGCTTCCCGG CAACAATTAA  
3481 TAGACTGGAT GGAGGCGGAT AAAGTTGCAG GACCACTTCT GCGCTCGGCC CTTCCGGCTG  
3541 GCTGGTTTAT TGCTGATAAA TCTGGAGCCG GTGAGCGTGG GTCTCGCGGT ATCATGTCAG  
3601 CACTGGGGCC AGATGGTAAG CCCTCCCGTA TCGTAGTTAT CTACACGAGC GGGAGTCAGG  
3661 CAACTATGGA TGAACGAAAT AGACAGATCG CTGAGATAGG TGCCTCACTG ATTAAGCATT  
3721 GGTAACGTG AGACCAAGTT TACTCATATA TACTTTAGAT TGATTTAAAA CTTCAITTTT  
3781 AATTTAAAAG GATCTAGGTG AAGATCCTTT TTGATAATCT CATGACCAAA ATCCCTTAAC  
3841 GTGAGTTTTT GTTCCACTGA GCGTCAGACC CCGTAGAAAA GATCAAAGGA TCTTCTTGAG  
3901 ATCCTTTTTT TCTGCGCGTA ATCTGCTGCT TGCAAACAAA AAAACCACCG CTACCAGCGG  
3961 TGGTTTGTIT GCCCGATCAA GAGCTACCAA CTCTTTTTCC GAAGGTAACCT GGCTTCAGCA  
4021 GAGCGCAGAT ACCAAATACT GTCCTTCTAG TGTAGCCGTA GTTAGGCCAC CACTTCAAGA  
4081 ACTCTGTAGC ACCGCCTACA TACCTCGCTC TGCTAATCCT GTTACCACTG GCTGCTGCCA  
4141 GTGGCGATAA GTCGTGTCTT ACCGGGTTGG ACTCAAGACG ATAGTTACCG GATAAGGCGC  
4201 AGCGGTTCGG CTGAACGGGG GGTTCGTGCA CACAGCCCAG CTTGGAGCGA ACGACCTACA  
4261 CCGAACTGAG ATACCTACAG CGTGAGCATT GAGAAAGCGC CACGCTTCCC GAAGGGAGAA  
4321 AGGCGGACAG GTATCCGGTA AGCGGCAGGG TCGGAACAGG AGAGCGCAGC AGGGAGCTTC  
4381 CAGGGGGAAA CGCCTGGTAT CTTTATAGTC CTGTGCGGTT TCGCCACCTC TGACTTGAGC  
4441 GTCGATTTTT GTGATGCTCG TCAGGGGGGC GGAGCCTATG GAAAAACGCC AGCAACGCGG  
4501 CCTTTTTACG GTTCTGGGCC TTTTGCTGGC CTTTGCTCA CATGTTCTTT CCTGCGTTAT  
4561 CCCCTGATTC TGTGGATAAC CGTATTACCG CTTTGAGTG AGCTGATACC GCTCGCCGCA  
4621 GCCGAACGAC CGAGCGCAGC GAGTCAGTGA GCGAGGAAGC GGAAGAGCGC CCAATACGCA  
4681 AACC GCCTCT CCCC GCCTG TGGCCGATTC ATTAATGCAG AGCTTGCAAT TCGCGCGCA  
4741 AGGCGAAGCG GCATTTACGT TGACACCATC GAATGGCGCA AAACCTTTCG CGGTATGGCA  
4801 TGATAGCGCC CGGAAGAGAG TCAATTCAGG GTGGTGAATG TGAAACCACT AACGTTATAC  
4861 GATGTCGCAG AGTATGCCGG TGTCTCTTAT CAGACCGTTT CCCGCGTGGT GAACAGGCC  
4921 AGCCACGTTT CTGCGAAAAC GCGGGAAAAA GTGGAAGCGG CGATGGCGGA GCTGAATTAC  
4981 ATTCCCAACC GCGTGGCACA ACAACTGGCG GGCAAACAGT CGTTGCTGAT TGGCGTTGCC  
5041 ACCTCCAGTC TGGCCCTGCA CGCGCCGTCG CAAATTGTCT GCGCGATTAA ATCTCGCGCC  
5101 GATCAACTGG GTGCCAGCGT GGTGGTGTCT ATGGTAGAAC GAAGCGCGCT CGAAGCCTGT  
5161 AAAGCGGCGG TGCACAATCT TCTCGCGCAA CGGGTCACTG GGCTGATCAT TAACTATCCG  
5221 CTGGATGACC AGGATGCCAT TGCTGTGGAA GCTGCCTGCA CTAATGTTCC GCGGTTATTT  
5281 CTTGATGTCT CTGACCAGAC ACCCATCAAC AGTATTATTT TCTCCCATGA AGACGGTACC  
5341 CGACTGGGCG TGGAGCATCT GGTCGCATTG GGTCAACAGC AAATCGCGCT GTTAGCGGGC  
5401 CCATTAAGTT CTGTCTCGCG GCGTCTGCGT CTGGCTGGCT GGCATAAATA TCTCACTCGC  
5461 AATCAAATTC AGCCGATAGC GGAACGGGAA GGCGACTGGA GTGCCATGTC CGGTTTTCAA  
5521 CAAACCATGC AAATGCTGAA TGAGGGCATC GTTCCCACTG CGATGCTGGT TGCCAACGAT  
5581 CAGATGGCGC TGGGCGCAAT GCGCGCCATT ACCGAGTCCG GGCTGCGCGT TGGTGCAGAT  
5641 ATCTCGGTAG TGGGATACGA CGATACCGAA GACAGCTCAT GTTATATCCC GCCGTCAACC  
5701 ACCATCAAAC AGGATTTTCG CCGTCTGGGG CAAACCAGCG TGGACCGCTT GCTGCAACTC  
5761 TCTCAGGGCC AGGCGGTGAA GGGCAATCAG CTGTTGCCCG TCTCACTGGT GAAAAGAAAA  
5821 ACCACCCTGG CGCCCAATAC GCAAACCGCC TCTCCCCGCG CGTTGGCCGA TTCATTAATG  
5881 CAGCTGGCAC GACAGGTTTC CCGACTGGAA AGCGGGCAGT GAGCGCAACG CAATTAATGT  
5941 GAGTTAGCTC ACTCATT

FIGURE 25D

Figure 26A

pDEST6

pSPORT " "  
(opposite strand)

"forward" sequencing primers

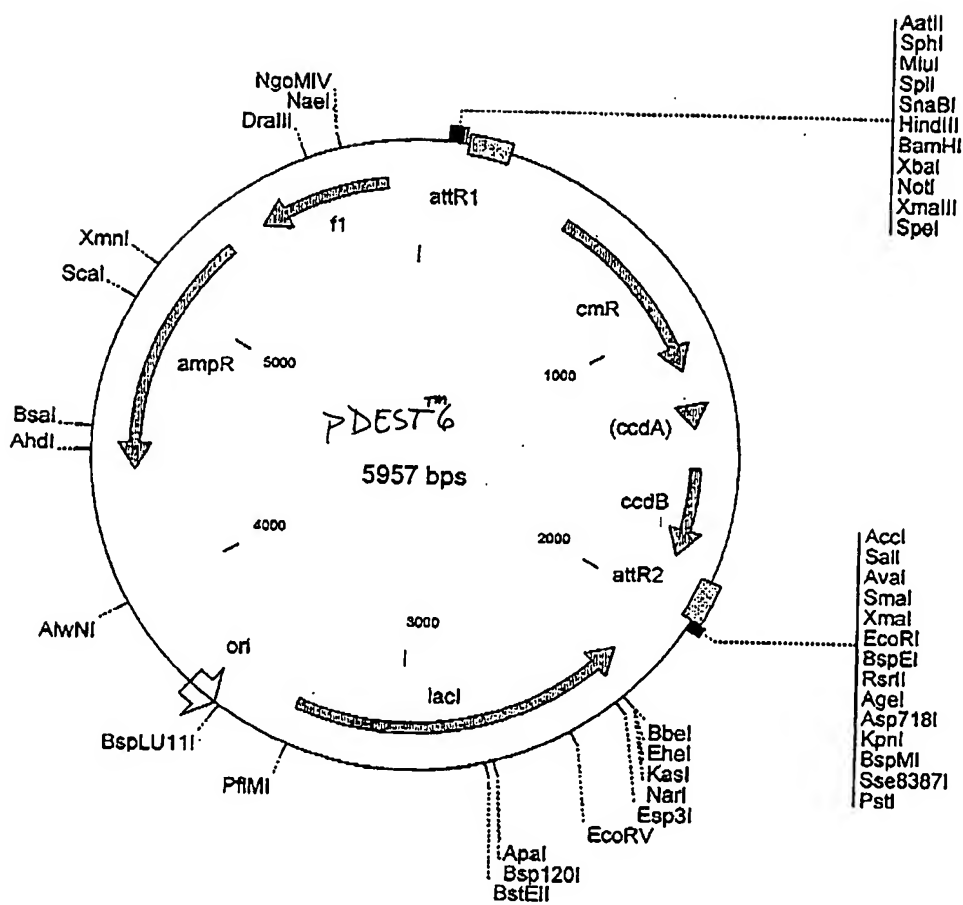
- 1 taa ~~cgc~~ cag ggt ttt ccc agt cac gac gtt gta aaa cga cgg cca gtg aat  
att gcg gtc cca aaa ggg tca gtg ctg caa cat ttt gct gcc ggt cac tta
- 52 tga att tag gtg aca cta tag aag agc tat gac gtc gca ~~tgt~~ ~~acg~~ cgt acg  
act taa atc cac tgt gat atc ttc tcg ata ctg cag ~~cgt~~ ~~acg~~ tgc gca tgc
- 103 ~~tca gct tgg atc ctc tag agc~~ ~~ggc cgc cga~~ ~~cta gtg atc~~ ~~aca agt tgg~~ ~~tac~~  
att cga ~~acc tag~~ ~~gag atc~~ ~~tcg ccg~~ ~~gcg gct gat~~ ~~gac tag~~ tgt tca aac atg
- 154 ~~aaa daa gct gaa cga gaa acg taa aat gar ata aat atc aat ata tta aat~~  
~~ttt tct cga ctt gct ctt tgc att tta cta tat tca tag tta tat aat tca~~
- ↓ Gene
- 1939 ~~tat tta tat tat ttt acg ttt cct gtt tag ctt~~ ~~tct tgt aca aag tgg tga~~  
~~ata aat ata gta daa tgc aaa gag aaa gtc gaa aga gca tgt~~ ttc acc att
- 1990 ~~tcg tcc acc cgg daa ttc cgg acc ggt act~~ ~~tgc agg cgt acc agc ttt~~ ccc  
agc agc ~~ggg gcc ctt aag gcc tgg~~ ~~dca tgg~~ ~~acg tcc gca tgg tgg~~ ~~aaa ggg~~
- T7 RNA
- 2041 tat agt gag tgc tat tag agc ttg gcg taa tca tgg tca tag ctg ttt cct  
ata tca ctc agc ata atc tgc aac cgc att agt acc agt atc gac aaa gga
- T7 promoter      α-peptide      "reverse .."
- 2092 gtg tga aat tgt tat ccg ctc aca att cca cac aac ata cga gct gga agc  
cac act tta aca ata ggc gag tgt taa ggt gtg tgg tad gct cgg cct tgc
- ... sequencing primers      lac RNA
- 2143 ~~ata aag~~ tgt aaa gcc tgg ggt gcc taa tga gtg agc taa ctc aca tta att  
tat ttc aca ttt cgg acc cca cgg att act cac tgc att gag tgt aat taa

53/240

Figure 26B

PDEST6

(cont'd)



54/240

## pDEST6 5957 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
266..142		attR1
516..1175		CmR
1295..1379		inactivated ccdA
1517..1822		ccdB
1863..1987		attR2
2203..3369		lacI
4403..5260		ampR
5392..5847		f1 (f1 intergenic region)
1	TAACGCCAGG GTTTTCCCAG TCACGACGTT GTAAAACGAC GGCCAGTGAA TTGAATTTAG	
61	GTGACACTAT AGAAGAGCTA TGACGTCGCA TGCACGCGTA CGTAAGCTTG GATCCTCTAG	
121	AGCGGCCGCC GACTAGTGAT CACAAGTTTG TACAAAAAAG CTGAACGAGA AACGTAAAAT	
181	GATATAAATA TCAATATATT AAATTAGATT TTGCATAAAA AACAGACTAC ATAATACTGT	
241	AAAACACAAC ATATCCAGTC ACTATGGCGG CCGCTAAGTT GGCAGCATCA CCCGACGCAC	
301	TTTGCGCCGA ATAAATACCT GTGACGGAAG ATCACTTCGC AGAATAAATA AATCCTGGTG	
361	TCCCTGTTGA TACCGGGAAG CCCTGGGCCA ACTTTTGGCG AAAATGAGAC GTTGATCGGC	
421	ACGTAAGAGG TTCCAACTTT CACCATAATG AAATAAGATC ACTACCGGGC GTATTTTTTTG	
481	AGTTATCGAG ATTTTCAGGA GCTAAGGAAG CTAAATGGA GAAAAAATC ACTGGATATA	
541	CCACCGTTGA TATATCCCAA TGGCATCGTA AAGAACATTT TGAGGCATTT CAGTCAGTTG	
601	CTCAATGTAC CTATAACCAG ACCGTTTCAGC TGGATATTAC GGCCTTTTTA AAGACCGTAA	
661	AGAAAAATAA GCACAAGTTT TATCCGGCCT TTATTCACAT TCTTGCCCCG CTGATGAATG	
721	CTCATCCGGA ATTCCGTATG GCAATGAAAG ACGGTGAGCT GGTGATATGG GATAGTGTTC	
781	ACCTTGTTA CACCGTTTTC CATGAGCAAA CTGAAACGTT TTCATCGCTC TGGAGTGAAT	
841	ACCACGACGA TTTCCGGCAG TTTCTACACA TATATTCGCA AGATGTGGCG TGTACGGTG	
901	AAAACCTGGC CTATTTCCCT AAAGGGTTTA TTGAGAATAT GTTTTTTCGT TCAGCCAATC	
961	CCTGGGTGAG TTTCACCACT TTTGATTTAA ACGTGCCAA TATGGACAAC TTCCTCGCCC	
1021	CCGTTTTTAC CATGGGCAAA TATTATACGC AAGGCGACAA GGTGCTGATG CCGCTGGCGA	
1081	TTCAGGTTCA TCATGCCGTC TGTGATGGCT TCCATGTCGG CAGAATGCTT AATGAATTAC	
1141	AACAGTACTG CGATGAGTGG CAGGGCGGGG CGTAAACGCG TGGATCCGGC TTAATAAAAG	
1201	CCAGATAACA GTATGCGTAT TTGCGCGCTG ATTTTTCGG TATAAGAATA TATACTGATA	
1261	TGTATACCCG AAGTATGTCA AAAAGAGGTG TGCTATGAAG CAGCGTATTA CAGTGACAGT	
1321	TGACAGCGAC AGCTATCAGT TGCTCAAGGC ATATATGATG TCAATATCTC CGGTCTGGTA	
1381	AGCACAAACA TGCAGAATGA AGCCCGTCGT CTGCGTGCCG AACGCTGGAA AGCGGAAAAT	
1441	CAGGAAGGGA TGGCTGAGGT CGCCCGGTTT ATTGAAATGA ACGGCTCTTT TGCTGACGAG	
1501	AACAGGGACT GGTGAAATGC AGTTTAAGGT TTACACCTAT AAAAGAGAGA GCCGTTATCG	
1561	TCTGTTTGTG GATGTACAGA GTGATATTAT TGACACGCCC GGGCGACGGA TGGTGATCCC	
1621	CCTGGCCAGT GCACGTCTGC TGTCAGATAA AGTCTCCCGT GAACTTTACC CGGTGGTGCA	
1681	TATCGGGGAT GAAAGCTGGC GCATGATGAC CACCGATATG GCCAGTGTGC CGGTCTCCGT	
1741	TATCGGGGAA GAAGTGGCTG ATCTCAGCCA CCGCGAAAAT GACATCAAAA ACGCCATTAA	
1801	CCTGATGTTC TGGGGAATAT AAATGTCAGG CTCCTTATA CACAGCCAGT CTGCAGGTG	
1861	ACCATAGTGA CTGGATATGT TGTGTTTTAC AGTATTATGT AGTCTGTTTT TTATGCAAAA	
1921	TCTAATTTAA TATATTGATA TTTATATCAT TTTACGTTTC TCGTTCAGCT TTCTGTACA	
1981	AAGTGGTGAT CGTCGACCCG GGAATTCGG ACCGGTACCT GCAGGCGTAC CAGCTTTCCC	
2041	TATAGTGAGT CGTATTAGAG CTGGCGTAA TCATGGTCAT AGCTGTTTCC TGTGTGAAAT	
2101	TGTTATCCGC TCACAATTCC ACACAACATA CGAGCCGGAA GCATAAAGTG TAAAGCCTGG	
2161	GGTGCCTAAT GAGTGAGCTA ACTCACATTA ATTGCGTTGC GCTCACTGCC CGCTTTCCAG	
2221	TCGGGAAACC TGTCGTGCCA GCTGCATTAA TGAATCGGCC AACGCGCGGG GAGAGGCGGT	
2281	TTGCGTATTG GGCGCCAGGG TGGTTTTTCT TTTACCAGT GAGACGGGCA ACAGCTGATT	
2341	GCCCTTCACC GCCTGGCCCT GAGAGAGTTG CAGCAAGCGG TCCACGCTGG TTTGCCCCAG	
2401	CAGGCGAAAA TCCTGTTTGA TGGTGGTTGA CGGCGGGATA TAACATGAG GTCTTCCGT	
2461	ATCGTCGTAT CCCACTACCG AGATATCCGC ACCAACGCGC AGCCCGGACT CGGTAATGGC	
2521	GCGCATTGCG CCCAGCGCCA TCTGATCGTT GGCAACCAGC ATCGCAGTGG GAACGATGCC	
2581	CTCATTCAGC ATTTGCATGG TTTGTTGAAA ACCGGACATG GCACTCCAGT CGCCTTCCCG	
2641	TTCCGCTATC GGCTGAATTT GATTGCGAGT GAGATATTTA TGCCAGCCAG CCAGACGCAG-	

FIGURE 26C

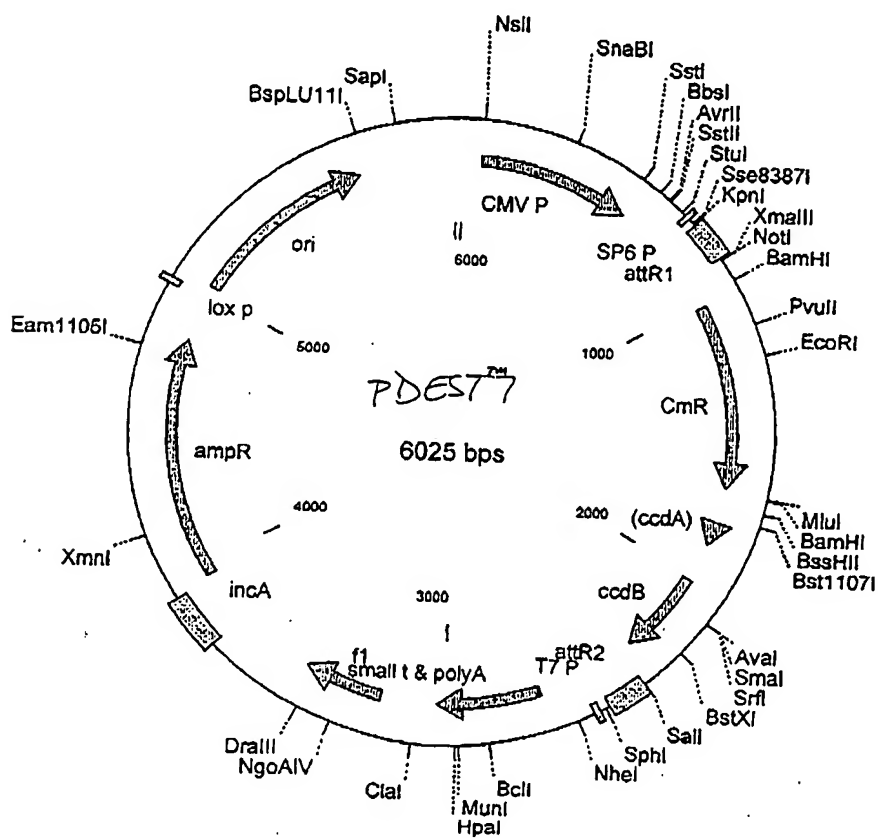
2701 ACGCGCCGAG ACAGAACTTA ATGGGCCCCG TAACAGCGCG ATTTGCTGGT GACCCAATGC  
2761 GACCAGATGC TCCACGCCCA GTCGCGTACC GTCTTCATGG GAGAAAATAA TACTGTTGAT  
2821 GGGTGTCTGG TCAGAGACAT CAAGAAATAA CGCCGGAACA TTAGTGCAGG CAGCTTCCAC  
2881 AGCAATGGCA TCCTGGTCAT CCAGCGGATA GTTAATGATC AGCCCACTGA CCGGTTGGCG  
2941 GAGAAGATTG TGCACCGCCG CTTTACAGGC TTCGACGCGG CTTCTGTTCTA CCATCGACAC  
3001 CACCACGCTG GCACCCAGTT GATCGGCGCG AGATTTAATC GCCGCGACAA TTTGCGACGG  
3061 CGCGTGCAGG GCCAGACTGG AGGTGGCAAC GCCAATCAGC AACGACTGTT TGCCCGCCAG  
3121 TTGTTGTGCC ACGCGGTTGG GAATGTAATT CAGCTCCGCC ATCGCCGCTT CCACTTTTTT  
3181 CCGCGTTTTT GCAGAAACGT GGCTGGCCTG GTTCACCACG CGGAAACGG TCTGATAAGA  
3241 GACACCGGCA TACTCTGCGA CATCGTATAA CGTTACTGGT TTCACATTCA CCACCTGAA  
3301 TTGACTCTCT TCCGGGCGCT ATCATGCCAT ACCGCGAAAG GTTTTTCGCC ATTCGATGGT  
3361 GTCAACGTAA ATGCCGCTTC GCCTTCGCGC GCGAATTGCA AGCTCTGCAT TAATGAATCG  
3421 GCCAACGCGC GGGGAGAGGC GGTTTTCGTA TTGGGCGCTC TTCCGCTTCC TCGCTCACTG  
3481 ACTCGCTGCG CTCGGTCGTT CGGCTGCGGC GAGCGGTATC AGCTCACTCA AAGGCGGTAA  
3541 TACGGTTATC CACAGAATCA GGGGATAACG CAGGAAAGAA CATGTGAGCA AAAGGCCAGC  
3601 AAAAGGCCAG GAACCGTAAA AAGGCCGCGT TGCTGGCGTT TTTCCATAGG CTCGCGCCCC  
3661 CTGACGAGCA TCACAAAAAT CGACGCTCAA GTCAGAGGTG GCGAAACCCG ACAGGACTAT  
3721 AAAGATACCA GCGGTTTTCC CCTGGAAGCT CCTCGTGCG CTCTCCTGTT CCGACCCTGC  
3781 CGCTTACCGG ATACCTGTCC GCCTTTCTCC CTTCCGGGAG CGTGGCGCTT TCTCAATGCT  
3841 CACGCTGTAG GTATCTCAGT TCGGTGTAGG TCGTTCGCTC CAAGCTGGGC TGTGTGCACG  
3901 AACCCCCCGT TCAGCCCGAC CGCTGCGCCT TATCCGGTAA CTATCGTCTT GAGTCCAACC  
3961 CGGTAAGACA CGACTTATCG CCACTGGCAG CAGCCACTGG TAACAGGATT AGCAGAGCGA  
4021 GGTATGTAGG CGGTGTACA GAGTCTTGA AGTGGTGGCC TAACTACGGC TACACTAGAA  
4081 GGACAGTATT TGGTATCTGC GCTCTGCTGA AGCCAGTTAC CTTGCGAAAA AGAGTTGGTA  
4141 GCTCTTGATC CGGCAAACAA ACCACCGCTG GTAGCGGTGG TTTTTTTGTT TGCAAGCAGC  
4201 AGATTACGCG CAGAAAAAAA GGATCTCAAG AAGATCCTTT GATCTTTTCT ACGGGGTCTG  
4261 ACGCTCAGTG GAACGAAAAA TCACGTAAAG GGATTTTGGT CATGAGATTA TCAAAAAGGA  
4321 TCTTCACCTA GATCCTTTTA AATTAAAAAT GAAGTTTTAA ATCAATCTAA AGTATATATG  
4381 AGTAAACTTG GTCTGACAGT TACCAATGCT TAATCAGTGA GGCACCTATC TCAGCGATCT  
4441 GTCTATTTTC TTCATCCATA GTTGCTGAC TCCCCGTCGT GTAGATAACT ACGATACGGG  
4501 AGGCTTACC ATCTGGCCCC AGTGCTGCAA TGATACCGCG AGACCCACGC TCACCGGCTC  
4561 CAGATTTATC AGCAATAAAC CAGCCAGCCG GAAGGGCCGA GCGCAGAAGT GGTCTTGCAA  
4621 CTTTATCCGC CTCCATCCAG TCTATTAATT GTTGCCGGGA AGCTAGAGTA AGTAGTTCCG  
4681 CAGTTAATAG TTTGCGCAAC GTTGTGCCA TTGCTACAGG CATCGTGGTG TCACGCTCGT  
4741 CGTTTGGTAT GGCTTCATTC AGCTCCGGTT CCCAACGATC AAGGCGAGTT ACATGATCCC  
4801 CCATGTTGTG CAAAAAAGCG GTTAGCTCCT TCGGTCTCCT GATCGTTGTC AGAAGTAAGT  
4861 TGGCCGCAGT GTTATCACTC ATGGTTATGG CAGCACTGCA TAATTCTCTT ACTGTCATGC  
4921 CATCCGTAAG ATGCTTTTCT GTGACTGGTG AGTACTCAAC CAAGTCATTC TGAGAATAGT  
4981 GTATGCGGCG ACCGAGTTGC TCTTGCCCGG CGTCAATACG GGATAATACC GCGCCACATA  
5041 GCAGAACTTT AAAAGTGCTC ATCATTGGAA AACGTTCTTC GGGGCGAAAA CTCTCAAGGA  
5101 TCTTACCGCT GTTGAGATCC AGTTCGATGT AACCCTACTC TGCACCCAAC TGATCTTCAG  
5161 CATCTTTTAC TTTACCAGC GTTCTGCGG GAGCAAAAAC AGGAAGGCAA AATGCCGCAA  
5221 AAAAGGGAAT AAGGGCGACA CGGAAATGTT GAATACTCAT ACTCTCCTT TTTCAATATT  
5281 ATTGAAGCAT TTATCAGGGT TATTGTCTCA TGAGCGGATA CATATTTGAA TGTATTTAGA  
5341 AAAATAAACA AATAGGGGTT CCGCGACAT TTCCCCGAAA AGTGCCACCT GAAATTGTAA  
5401 ACGTTAATAT TTTGTTAAAA TTCGCGTTAA ATTTTGTGTA AATCAGCTCA TTTTTTAAAC  
5461 AATAGGCCGA AATCGGCAAA ATCCCTTATA AATCAAAAGA ATAGACCGAG ATAGGGTTGA  
5521 GTGTTGTTCC AGTTTGGAAC AAGAGTCCAC TATTAAAGAA CGTGGACTCC AACGTCAAAG  
5581 GGCAGAAAAC CGTCTATCAG GCGGATGGCC CACTACGTGA ACCATCACCC TAATCAAGTT  
5641 TTTTGGGGTC GAGGTGCCGT AAAGCACTAA ATCGGAACCC TAAAGGGAGC CCCCATTATA  
5701 GAGCTTGACG GGGAAAGCCG GCGAAACGTG CGAGAAAGGA AGGGAAGAAA GCGAAAGGAG  
5761 CGGGCGCTAG GCGCTGGCA AGTGTAGCGG TCACGCTGCG CGTAACCACC ACACCCGCCG  
5821 CGCTTAATGC GCCGCTACAG GCGCGTCCA TTCGCCATTG AGGCTGCGCA ACTGTTGGGA  
5881 AGGGCGATCG GTGCGGCCT CTTGCTATT ACGCCAGCTG GCGAAAGGGG GATGTGCTGC  
5941 AAGGCGATTA AGTTGGG

FIGURE 26D

Figure 27A: PDEST7

## CMV promoter for eukaryotic expression

970 cca ttg acg caa atg ggc ggt agg cgt gta cgg tgg gag gtc tat ata agc  
 ggt aac tgc gtt tac ccg cca tcc gca cat gcc acc ctc cag ata tat tcc  
 1021 aga gct cgt tta gtg aac cgt cag atc gcc tgg aga cgc cat cca cgc tgt  
 tct cga gca aat cac ttg gca gtc tag cgg acc tct gcg gta ggt gcg aca  
 CMV enhancer / promoter  
 1072 ttt gac ctc cat aga aga cac cgg gac cga tcc agc ctc cgt act cta gcc  
 aaa ctg gag gta tct tct gtg gcc ctg gct agg tcc gag ggc tga gat cgg  
 1123 tag gcc gcg gag cgg ata aca att tca cac agg aaa cag cta tga cca cta  
 atc cgg cgc ctc gcc tat tgt taa agt gtg tcc ttt gtc gat act ggt gat  
 1174 ggc ttt tgc aaa aag cta ttt agg tga cac tat aga agg tac gcc tgc agc  
 ccg aaa acg ttt ttc gat aaa tcc act gtg ata tct tcc atg cgg acg tcc  
 1225 <sup>Kpn</sup> <sup>EcoRI</sup> <sup>NotI</sup> <sup>AttR1</sup> <sup>Pst</sup>  
 tac cgg tcc gga att ccc atc aca agt tgg tag aag aag ggt gaa cga gaa  
 atg gcc agg cct taa ggg tag tgt tca aac atg ttt tct cga cta gct cct



## pDEST7 6025 bp (rotated to position 2800)

<u>Location (Base Nos.)</u>			<u>Gene Encoded</u>			
	67..589		CMV promoter			
	906..782		attR1			
	1015..1674		CmR			
	1794..1878		inactivated ccdA			
	2016..2321		ccdB			
	2362..2486		attR2			
	2671..3033		small t & polyA			
	3227..3502		f1			
	3962..4822		ampR			
	5022..5661		ori			
1	ATTATCATGA	CATTAACCTA	TAAAAATAGG	CGTAGTACGA	GGCCCTTTCA	CTCATTAGAT
61	GCATGTCGTT	ACATAACTTA	CGGTAATAGG	CCCCGCTGGC	TGACCGCCCA	ACGACCCCCG
121	CCCATTTGACG	TCAATAATGA	CGTATGTTCC	CATAGTAACG	CCAATAGGGA	CTTTCCATTG
181	ACGTCAATGG	GTGGAGTATT	TACGGTAAAC	TGCCCCACTTG	GCAGTACATC	AAGTGTATCA
241	TATGCCAAGT	ACGCCCCCTA	TTGACGTCAA	TGACGGTAAA	TGGCCCCGCT	GGCATTATGC
301	CCAGTACATG	ACCTTATGGG	ACTTTCCTAC	TTGGCAGTAC	ATCTACGTAT	TAGTCATCGC
361	TATTACCATG	GTGATGCGGT	TTTGGCAGTA	CATCAATGGG	CGTGGATAGC	GGTTTGACTC
421	ACGGGGATTT	CCAAGTCTCC	ACCCCATTTGA	CGTCAATGGG	AGTTTGTTTT	GGCACCAAAA
481	TCAACGGGAC	TTTCCAAAAT	GTGTAACAA	CTCCGCCCCA	TTGACGCAAA	TGGGCGGTAG
541	GCGTGTACGG	TGGGAGGTCT	ATATAAGCAG	AGCTCGTTTA	GTGAACCGTC	AGATCGCCTG
601	GAGACGCCAT	CCACGCTGTT	TTGACCTCCA	TAGAAGACAC	CGGGACCGAT	CCAGCCTCCG
661	GACTCTAGCC	TAGGCCGCGG	AGCGGATAAC	AATTTACAC	AGGAAACAGC	TATGACCATT
721	AGGCCTTTGC	AAAAAGCTAT	TTAGGTGACA	CTATAGAAGG	TACGCCTGCA	GGTACCGGAT
781	CACAAGTTTG	TACAAAAAAG	CTGAACGAGA	AACGTAAAAT	GATATAAATA	TCAATATATT
841	AAATTAGATT	TTGCATAAAA	AACAGACTAC	ATAATACTGT	AAAACACAAC	ATATCCAGTC
901	ACTATGGCGG	CCGCATTAGG	CACCCAGGC	TTTACACTTT	ATGCTTCCGG	CTCGTATAAT
961	GTGTGGATTT	TGAGTTAGGA	TCCGTCGAGA	TTTTCAGGAG	CTAAGGAAGC	TAAAATGGAG
1021	AAAAAAATCA	CTGGATATAC	CACCGTTGAT	ATATCCCAAT	GGCATCGTAA	AGAACATTTT
1081	GAGGCATTTT	AGTCAGTTGC	TCAATGTACC	TATAACCAGA	CCGTTTCAGT	GGATATTACG
1141	GCCTTTTTAA	AGACCGTAAA	GAAAAATAAG	CACAAGTTTT	ATCCGGCCTT	TATTCACATT
1201	CTTGCCCGCC	TGATGAATGC	TCATCCGGAA	TTCCGTATGG	CAATGAAAGA	CGGTGAGCTG
1261	GTGATATGGG	ATAGTGTTCA	CCCTTGTTAC	ACCGTTTTCC	ATGAGCAAAC	TGAAACGTTT
1321	TCATCGCTCT	GGAGTGAATA	CCACGACGAT	TTCCGGCAGT	TTCTACACAT	ATATTGCGAA
1381	GATGTGGCGT	GTTACGGTGA	AAACCTGGCC	TATTTCCCTA	AAGGGTTTAT	TGAGAATATG
1441	TTTTTCGTCT	CAGCCAATCC	CTGGGTGAGT	TTCAACAGTT	TTGATTTAAA	CGTGGCCAAT
1501	ATGGACAAC	TCTTCGCCCC	CGTTTTTACC	ATGGGCAAA	ATTATACGCA	AGGCGACAAG
1561	GTGCTGATGC	CGCTGGCGAT	TCAGGTTTCT	CATGCCGTCT	GTGATGGCTT	CCATGTCGGC
1621	AGAATGCTTA	ATGAATTACA	ACAGTACTGC	GATGAGTGGC	AGGGCGGGGC	GTAAACGCGT
1681	GGATCCGGCT	TACTAAAAGC	CAGATAACAG	TATGCGTATT	TGCGCGCTGA	TTTTTGCGGT
1741	ATAAGAATAT	ATACTGATAT	GTATACCCGA	AGTATGTCAA	AAAGAGGTGT	GCTATGAAGC
1801	AGCGTATTAC	AGTGACAGTT	GACAGCGACA	GCTATCAGTT	GCTCAAGGCA	TATATGATGT
1861	CAATATCTCC	GGTCTGGTAA	GCACAACCAT	GCAGAATGAA	GCCCGTCGTC	TGCGTGCCGA
1921	ACGCTGGA	GCGGAAAATC	AGGAAGGGAT	GGCTGAGGTC	GCCCGGTTTA	TTGAAATGAA
1981	CGGCTCTTTT	GCTGACGAGA	ACAGGGACTG	GTGAAATGCA	GTTTAAGGTT	TACACCTATA
2041	AAAGAGAGAG	CCGTTATCGT	CTGTTTGTGG	ATGTACAGAG	TGATATTATT	GACACGCCCC
2101	GGCGACGGAT	GGTGATCCCC	CTGGCCAGTG	CACGCTGCTG	GTCAGATAAA	GTCTCCCGTG
2161	AACTTTACCC	GGTGGTGATC	ATCGGGGATG	AAAGCTGGCG	CATGATGACC	ACCGATATGG
2221	CCAGTGTGCC	GGTCTCCGTT	ATCGGGGAAG	AAGTGGCTGA	TCTCAGCCAC	CGCGAAAATG
2281	ACATCAAAAA	CGCCATTAA	CTGATGTTCT	GGGGAATATA	AATGTCAGGC	TCCCTTATAC
2341	ACAGCCAGTC	TGCAGGTCTA	CCATAGTGAC	TGGATATGTT	GTGTTTACA	GTATTATGTA
2401	GTCTGTTTTT	TATGCAAAAT	CTAATTTAAT	ATATTGATAT	TTATATCATT	TTACGTTTCT
2461	CGTTTCAGCTT	TCTTGTAACA	AGTGGTGATC	CGGTGCATGC	GACGTCAATG	CTCTCTCCCT
2521	ATAGTGAGTC	GTATTATAAG	CTAGGCACTG	GCCGTCGTTT	TACAACGTCG	TGACTGGGAA

FIGURE 27B

2581 AACTGCTAGC TTGGGATCTT TGTGAAGGAA CCTTACTTCT GTGGTGTGAC ATAATTGGAC  
2641 AAACCTACCTA CAGAGATTTA AAGCTCTAAG GTAAATATAA AATTTTTAAG TGTATAATGT  
2701 GTTAAACTAG CTGCATATGC TTGCTGCTTG AGAGTTTTGC TTAGCTAGTA TGATTTATGA  
2761 AAATATTATA CACAGGAGCT AGTGATTCTA ATTGTTTG TG TATTTTAGAT TCACAGTCCC  
2821 AAGGCTCATT TCAGGCCCTT CAGTCTCTAC AGTCTGTTCA TGATCATAAT CAGCCATACC  
2881 ACATTTGTAG AGGTTTTACT TGCTTTAAAA AACCTCCAC ACCTCCCCCT GAACCTGAAA  
2941 CATAAAATGA ATGCAATTGT TGTGTTAAAC TTGTTTATTG CAGCTTATAA TGGTTACAAA  
3001 TAAAGCAATA GCATCACAAA TTTCACAAAT AAAGCATTTT TTCACTGCA TTCTAGTTGT  
3061 GGTTTGTCCA AACTCATCAA TGTATCTTAT CATGTCTGGA TCGATCCTGC ATTAATGAAT  
3121 CGGCCAACGC GCGGGGAGAG GCGGTTTGCG TATTGGCTGG CGTAATAGCG AAGAGGCCCG  
3181 CACCGATCGC CCTTCCCAAC AGTTGCGCAG CCTGAATGGC GAATGGGACG CGCCCTGTAG  
3241 CGGCGCATTG AGCGCGGCGG GTGTGGTGGT TACGCGCAGC GTGACCGCTA CACTTGCCAG  
3301 CGCCCTAGCG CCCGCTCCTT TCGCTTTCTT CCCTTCCTTT CTCGCCACGT TCGCCGGCTT  
3361 TCCCCGTCAA GCTCTAAATC GGGGGCTCCC TTTAGGGTTC CGATTTAGTG CTTTACGGCA  
3421 CCTCGACCCC AAAAACTTG ATTAGGGTGA TGGTTCACGT AGTGGGCCAT CGCCCTGATA  
3481 GACGGTTTTT CGCCCTTGA CGTTGGAGTC CACGTTCTTT AATAGTGGAC TCTGTTCGA  
3541 AACTGGAACA ACACTCAACC CTATCTCGGT CTATTTCTTT GATTTATAAG GGATTTTGCC  
3601 GATTTGCGCC TATTGGTTAA AAAATGAGCT GATTTAACA AAATTTAAG CGAATTTTAA  
3661 CAAAATATTA ACGTTTACAA TTTCAGGTGG CACTTTTCGG GGAAATGTGC GCGGAACCCC  
3721 TATTTGTTTA TTTTCTAAA TACATTCAA TATGTATCCG CTCATGCCAG GTCTTGGACT  
3781 GGTGAGAACG GCTTGCTCGG CAGCTTCGAT GTGTGCTGGA GGGAGAATA AGGTCTAAGA  
3841 TGTGCGATAG AGGGAAGTCG CATTTGAATTA TGTGCTGTGT AGGGATCGT GGTATCAAAT  
3901 ATGTGTGCCC ACCCCTGGCA TGAGACAATA ACCCTGATA ATGCTTCAAT AATATTGAAA  
3961 AAGGAAGAGT ATGAGTATTC AACATTTCCG TGTGCGCCTT ATTCCCTTTT TTGCGGCATT  
4021 TTGCCTTCTT GTTTTTGCTC ACCCAGAAAC GCTGGTGAAA GTAAAAGATG CTGAAGATCA  
4081 GTTGGGTGCA CGAGTGGGTT ACATCGAACT GGATCTCAAC AGCGGTAAGA TCCTTGAGAG  
4141 TTTTCGCCCC GAAGAACGTT TTCCAATGAT GAGCACTTTT AAAGTTCTGC TATGTGGCGC  
4201 GGTATTATCC CGTATTGACG CCGGGCAAGA GCAACTCGGT CGCCGCATAC ACTATTCTCA  
4261 GAATGACTTG GTTGAGTACT CACCAGTCAC AGAAAAGCAT CTTACGGATG GCATGACAGT  
4321 AAGAGAATTA TGCAGTGCTG CCATAACCAT GAGTGATAAC ACTGCGGCCA ACTTACTTCT  
4381 GACAACGATC GGAGGACCGA AGGAGCTAAC CGCTTTTTTG CACAACATGG GGGATCATGT  
4441 AACTCGCCTT GATCGTTGGG AACCGGAGCT GAATGAAGCC ATACCAAACG ACCGAGCGTGA  
4501 CACCACGATG CCTGTAGCAA TGGCAACAAC GTTGGCAGAA CTATTAACCT GCGAACTACT  
4561 TACTCTAGCT TCCCGGCAAC AATTAATAGA CTGGATGGAG GCGGATAAAG TTGCAAGACC  
4621 ACTTCTGCGC TCGGCCCTTC CGGCTGGCTG GTTTATTGCT GATAAATCTG GAGCCGGTGA  
4681 GCGTGGGTCT CGCGGTATCA TTGCAGCACT GGGGCCAGAT GGTAAGCCCT CCCGTATCGT  
4741 AGTTATCTAC ACGACGGGGA GTGAGGCAAC TATGGATGAA CGAAATAGAC AGATCGCTGA  
4801 GATAGGTGCC TCACTGATTA AGCATTGGTA ACTGTCAGAC CAAGTTTACT CATATATACT  
4861 TTAGATTGAT TTAAACTTTC ATTTTAAAT TAAAAGGATC TAGGTGAAGA TCCTTTTGA  
4921 TAATCTCATG CCATAACTTC GTATAATGTA TGCTATACGA AGTTATGGCA TGACCAAAAT  
4981 CCCTTAACGT GAGTTTTCGT TCCACTGAGC GTCAGACCCC GTAGAAAAGA TCAAAGGATC  
5041 TTCTTGAGAT CCTTTTTTTC TGC CGTAAT CTGCTGCTTG CAAACAAAAA AACCACCGCT  
5101 ACCAGCGGTG GTTTGTTTGC CGGATCAAGA GCTACCAACT CTTTTTCCGA AGGTAACCTG  
5161 CTTACAGAGA GCGCAGATAC CAAATACTGT CCTTCTAGTG TAGCCGTAGT TAGGCCACCA  
5221 CTTCAAGAAC TCTGTAGCAC CGCCTACATA CCTCGCTCTG CTAATCCTGT TACCAGTGGC  
5281 TGCTGCCAGT GCGGATAAGT CGTGTCTTAC CGGGTTGGAC TCAAGACGAT AGTTACCGGA  
5341 TAAGGCGCAG CGGTCGGGCT GAACGGGGGG TTCGTGCACA CAGCCCAGCT TGGAGCGAAC  
5401 GACCTACACC GAAGTGAAT ACCTACAGCG TGAGCATTGA GAAAGCGCCA CGCTTCCCGA  
5461 AGGGAGAAAG GCGGACAGGT ATCCGGTAAG CGGAGGGTTC GGAACAGGAG AGCGCACGAG  
5521 GGAGCTTCCA GGGGGAACG CCTGGTATCT TTATAGTCTT GTGCGGTTTC GCCACCTCTG  
5581 ACTTGAGCGT CGATTTTGTG GATGCTCGTC AGGGGGGCGG AGCCTATGGA AAAACGCCAG  
5641 CAACGCGGCC TTTTACGGT TCCTGGCCTT TTGCTGGCCT TTTGCTCACA TGTTCTTTCC  
5701 TCGGTTATCC CCTGATTCTG TGGATAACCG TATTACCGCC TTTGAGTGAG CTGATACCGC  
5761 TCGCCGACG CGAACGACCG AGCGCAGCGA GTCAGTGAGC GAGGAAGCGG AAGAGCGCCC  
5821 AATACGCAAA CCGCCTCTCC CCGCGGTTG GCCGATTCT TAATGCAGAG CTGCAATTC  
5881 GCGCGTTTTT CAATATTATT GAAGCATTTA TCAGGGTTAT TGTCTCATGA GCGGATACAT  
5941 ATTTGAATGT ATTTAGAAAA ATAAACAAAT AGGGGTTCCG CGCACATTTT CCGGAAAAGT  
6001 GCCACCTGAC GTCTAAGAAA CCATT



Figure 28A: pDEST8 Polyhedron Promoter, Baculovirus Transfer Plasmid

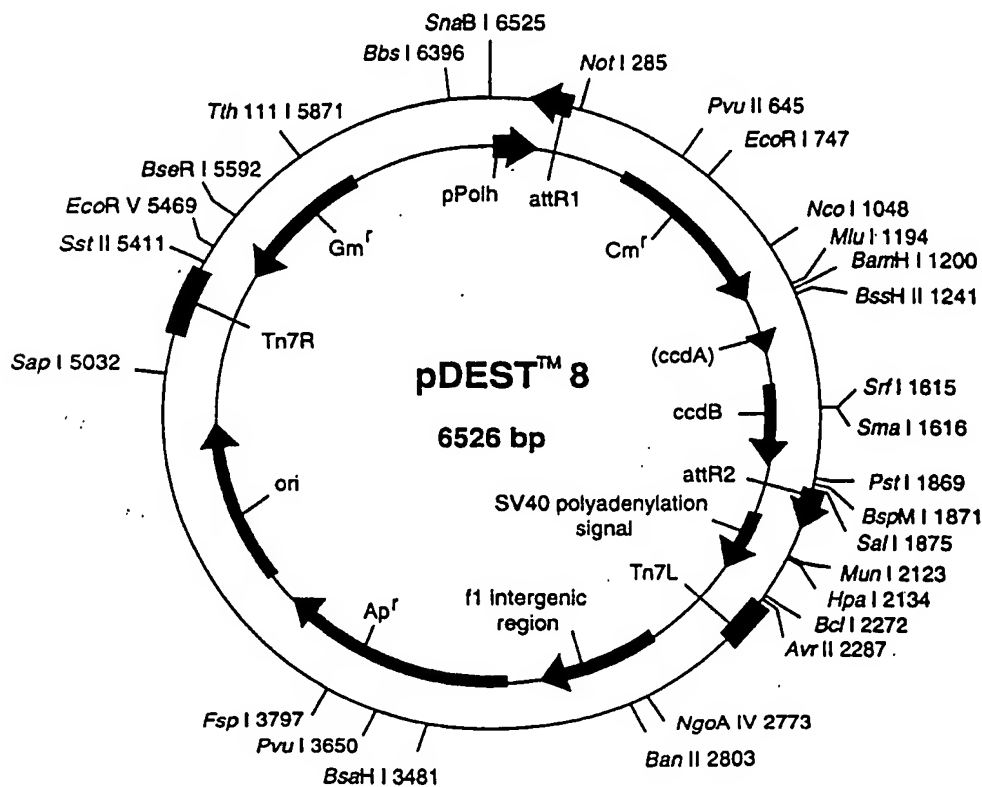
**Ace I**

```

1  cgt|ata ctc cgg aat att aat aga tca tgg aga taa tta aaa tga taa cca
   gca tat gag gcc tta taa tta tct agt acc tct att aat ttt act att ggt
52  tct cgc aaa taa ata → mRNA (polyhedrin) agt att tta ctg ttt tgg taa cag ttt tgt aat aaa
   aga gcg ttt att tat tca taa aat gac aaa agc att gtc aaa aca tta ttt
103 aaa acc tat aaa tat tcc gga tta ttc ata ccg tcc cac cat cgg gcg cgg
   ttt tgg ata ttt ata agg cct aat aag tat ggc agg gtg gta gcc cgc gcc
154 atc|atc aca agt tgg tgg aaa aaa gct gaa cga gaa aag taa dat gat ata
   tag tag tgt tca aac atg ttt ttc cga ctt gct ctt tgc att tta ctg tat

```

**Bam**



60/240

## pDEST8 6526 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
23..152		Ppolh
284..160		attR1
534..1193		CmR
1313..1397		inactivated ccdA
1535..1840		ccdB
1881..2005		attR2
2766..3146		f1
3240..4090		ampR
4289..4869		ori
5564..6496		genR

1	CGTATACTCC	GGAATATTAA	TAGATCATGG	AGATAATTAA	AATGATAACC	ATCTCGCAAA
61	TAAATAAGTA	TTTTACTGTT	TTCGTAACAG	TTTTGTAAATA	AAAAAACCTA	TAAATATTCC
121	GGATTATTCA	TACCGTCCCA	CCATCGGGCG	CGGATCATCA	CAAGTTTGTA	CAAAAAAGCT
181	GAACGAGAAA	CGTAAATGA	TATAAATATC	AATATATTAA	ATTAGATTTT	GCATAAAAAA
241	CAGACTACAT	AATACTGTAA	AACACAACAT	ATCCAGTCAC	TATGGCGGCC	GCTAAGTTGG
301	CAGCATCACC	CGACGCACTT	TGCGCCGAAT	AAATACCTGT	GACGGAAGAT	CACTTCGCAG
361	AATAAATAAA	TCCTGGTGTC	CCTGTTGATA	CCGGGAAGCC	CTGGGCCAAC	TTTTGGCGAA
421	AATGAGACGT	TGATCGGCAC	GTAAGAGGTT	CCAACTTTCA	CCATAATGAA	ATAAGATCAC
481	TACCGGGCGT	ATTTTTTGAG	TTATCGAGAT	TTTCAGGAGC	TAAGGAAGCT	AAAATGGAGA
541	AAAAAATCAC	TGGATATACC	ACCGTTGATA	TATCCCAATG	GCATCGTAAA	GAACATTTTG
601	AGGCATTTCA	GTCAGTTGCT	CAATGTACCT	ATAACCAGAC	CGTTCAGCTG	GATATTACGG
661	CCTTTTTTAA	GACCGTAAAG	AAAAATAAGC	ACAAGTTTTA	TCCGGCCTTT	ATTCACATTC
721	TTGCCCGCCT	GATGAATGCT	CATCCGGAAT	TCCGTATGGC	AATGAAAGAC	GGTGAGCTGG
781	TGATATGGGA	TAGTGTTCAC	CCTTGTTACA	CCGTTTTCCA	TGAGCAAATC	GAACCGTTTT
841	CATCGCTCTG	GAGTGAATAC	CACGACGATT	TCCGGCAGTT	TCTACACATA	TATTCGCAAG
901	ATGTGGCGTG	TTACGGTGAA	AACCTGGCCT	ATTTCCCTAA	AGGGTTTATT	GAGAATATGT
961	TTTTCGTCTC	AGCCAATCCC	TGGGTGAGTT	TCACCAGTTT	TGATTTAAAC	GTGGCCAATA
1021	TGGACAACCT	CTTCGCCCCC	GTTTTACCA	TGGGCAAATA	TTATACGCAA	GGCGACAAGG
1081	TGCTGATGCC	GCTGGCGATT	CAGGTTTCATC	ATGCCGCTCTG	TGATGGCTTC	CATGTCGGCA
1141	GAATGCTTAA	TGAATTACAA	CAGTACTGCG	ATGAGTGGCA	GGGCGGGGCG	TAAACGCGTG
1201	GATCCGGCTT	ACTAAAAGCC	AGATAACAGT	ATGCGTATTT	GCGCGCTGAT	TTTTGCGGTA
1261	TAAGAATATA	TACTGATATG	TATACCCGAA	GTATGTCAAA	AAGAGGTGTG	CTATGAAGCA
1321	GCGTATTACA	GTGACAGTTG	ACAGCGACAG	CTATCAGTTG	CTCAAGGCAT	ATATGATGTC
1381	AATATCTCCG	GTCTGGTAAG	CACAACCATG	CAGAATGAAG	CCCGTCTGCT	GCCTGCCGAA
1441	CGCTGGAAAG	CGGAAAATCA	GGAAGGGATG	GCTGAGGTCG	CCCGGTTTAT	TGAAATGAAC
1501	GGCTCTTTTG	CTGACGAGAA	CAGGGACTGG	TGAAATGCAG	TTTAAGGTTT	ACACCTATAA
1561	AAGAGAGAGC	CGTTATCGTC	TGTTTGTGGA	TGTACAGAGT	GATATTATTG	ACACGCCCGG
1621	GCGACGGATG	GTGATCCCCC	TGGCCAGTGC	ACGTCTGCTG	TCAGATAAAG	TCTCCCGTGA
1681	ACTTTACCCG	GTGGTGCATA	TCGGGGATGA	AAGCTGGCGC	ATGATGACCA	CCGATATGGC
1741	CAGTGTGCCG	GTCTCCGTTA	TCGGGGAAGA	AGTGGCTGAT	CTCAGCCACC	GCGAAAATGA
1801	CATCAAAAAC	GCCATTAACC	TGATGTTCTG	GGGAATATAA	ATGTCAGGCT	CCCTTATACA
1861	CAGCCAGTCT	GCAGGTCGAC	CATAGTGACT	GGATATGTTG	TGTTTTACAG	TATTATGTAG
1921	TCTGTTTTTT	ATGCAAAATC	TAATTTAATA	TATGTATATT	TATATCATTT	TACGTTTCTC
1981	GTTCAGCTTT	CTTGTAACAA	GTGGTGATAG	CTTGTCGAGA	AGTACTAGAG	GATCATAATC
2041	AGCCATACCA	CATTTGTAGA	GGTTTACTTT	GCTTTAAAAA	ACCTCCACAC	CCTCCCCCTG
2101	AACCTGAAAC	ATAAAATGAA	TGCAATTGTT	GTTGTTAACT	TGTTTTATTG	AGCTTATAAT
2161	GGTTACAAAT	AAAGCAATAG	CATCACAAT	TTCAACAAATA	AAGCATTTTT	TTCACTGCAT
2221	TCTAGTTGTG	GTTTGTCCAA	ACTCATCAAT	GTATCTTATC	ATGTCTGGAT	CTGATCACTG
2281	CTTGAGCCTA	GGAGATCCGA	ACCAGATAAG	TGAAATCTAG	TTCCAAACTA	TTTTGTCAAT
2341	TTTAATTTTC	GTATTAGCTT	ACGACGCTAC	ACCCAGTTCC	CATCTATTTT	GTCACTCTTC
2401	CCTAAATAAT	CCTTAAAAAC	TCCATTTCCA	CCCCCTCCAG	TTCCCAACTA	TTTTGTCCGC
2461	CCACAGCGGG	GCATTTTCTT	TCCTGTTATG	TTTTTAATCA	AACATCCTGC	CAACTCCATG
2521	TGACAAACCG	TCATCTTCGG	CTACTTTTTTC	TCTGTCACAG	AATGAAAATT	TTTCTGTCAT

FIGURE 28B

2581 CTCTTCGTTA TTAATGTTTG TAATTGACTG AATATCAACG CTTATTGCA GCCTGAATGG  
2641 CGAATGGACG CGCCCTGTAG CGGCGCATTA AGCGCGGCGG GTGTGGTGGT TACGCGCAGC  
2701 GTGACCGCTA CACTTGCCAG CGCCCTAGCG CCCGCTCCTT TCGCTTTCTT CCCTTCCTTT  
2761 CTCGCCACGT TCGCCGCGTT TCCCCGTCAA GCTCTAAATC GGGGGCTCCC TTTAGGGTTC  
2821 CGATTTAGTG CTTTACGGCA CCTCGACCCC AAAAACTTG ATTAGGGTGA TGGTTCACGT  
2881 AGTGGGCCAT CGCCCTGATA GACGGTTTTT CGCCCTTGA CGTTGGAGTC CACGTTCTTT  
2941 AATAGTGGAC TCTTGTTCCA AACTGGAACA ACACTCAACC CTATCTCGGT CTATTCTTTT  
3001 GATTTATAAG GGATTTTGCC GATTTGCGCC TATTGGTTAA AAAATGAGCT GATTTAACAA  
3061 AAATTTAACG CGAATTTTAA CAAAATATTA ACGTTTACAA TTTCAGGTGG CACTTTTCGG  
3121 GGAATGTGTC GCGGAACCCC TATTTGTTTA TTTTCTAAA TACATTCAA TATGTATCCG  
3181 CTCATGAGAC AATAACCTG ATAAATGCTT CAATAATATT GAAAAAGAA GAGTATGAGT  
3241 ATTCAACATT TCCGTGTCGC CCTTATTCCC TTTTTCGCG CATTTTGGCT TCCTGTTTTT  
3301 GCTACCCAG AAACGCTGGT GAAAGTAAAA GATGCTGAAG ATCAGTTGGG TGCACGAGTG  
3361 GGTTACATCG AACTGGATCT CAACAGCGGT AAGATCCTTG AGAGTTTTCG CCCCAGAGAA  
3421 CGTTTTCCAA TGATGAGCAC TTTTAAAGTT CTGCTATGTG GCGCGGTATT ATCCCGTATT  
3481 GACGCGGGC AAGAGCAACT CGGTGCGCGC ATACACTATT CTCAGAATGA CTTGGTTGAG  
3541 TACTCACCAG TCACAGAAAA GCATCTTACG GATGGCATGA CAGTAAGAGA ATTATGCAGT  
3601 GCTGCCATAA CCATGAGTGA TAACACTGCG GCCAACTTAC TTCTGACAAAC GATCGGAGGA  
3661 CCGAAGGAGC TAACCGCTTT TTTGCACAAC ATGGGGGATC ATGTAACGCG CTTGATCGT  
3721 TGGGAACCGG AGCTGAATGA AGCCATACCA AACGACGAGC GTGACACCAC GATGCTGTGA  
3781 GCAATGGCAA CAACGTTGCG CAACTATTA ACTGGCGAAC TACTTACTCT AGCTTCCCGG  
3841 CAACAATTA TAGACTGGAT GGAGGCGGAT AAAGTTGCAG GACCACTTCT GCGCTCGGCC  
3901 CTTCCGGCTG GCTGGTTTAT TGCTGATAAA TCTGGAGCCG GTGAGCGTGG GTCTCGCGGT  
3961 ATCATTGCAG CACTGGGGCC AGATGGTAAG CCCTCCCGTA TCGTAGTTAT CTACACGACG  
4021 GGGAGTCAGG CAACTATGGA TGAACGAAAT AGACAGATCG CTGAGATAGG TGCCTCACTG  
4081 ATTAAGCATT GGTAAGTGTC AGACCAAGTT TACTCATATA TACTTTAGAT TGATTTAAAA  
4141 CTTCATTTTT AATTTAAAG GATCTAGGTG AAGATCCTTT TTGATAATCT CATGACCAAA  
4201 ATCCCTTAAC GTGAGTTTTT GTTCCACTGA GCGTCAGACC CCGTAGAAAA GATCAAAGGA  
4261 TCTTCTTGAG ATCCTTTTTT TCTGCGCGTA ATCTGCTGCT TGCAACAAAA AAAACACCG  
4321 CTACCAGCGG TGGTTTGTGTT GCCGATCAA GAGCTACCAA CTCTTTTTTC GAAGGTAAC  
4381 GGCTTCAGCA GAGCGCAGAT ACCAAATACT GTCCTTCTAG TGTAGCCGTA GTTAGGCCAC  
4441 CACTTCAAGA ACTCTGTAGC ACCGCCTACA TACCTCGCTC TGCTAATCCT GTTACCAGTG  
4501 GCTGCTGCCA GTGGCGATAA GTCGTGCTT ACCGGGTGG ACTCAAGACG ATAGTTACCG  
4561 GATAAGGCGC AGCGGTGCGG CTGAACGGGG GGTTCGTGCA CACAGCCCAG CTTGGAGCGA  
4621 ACGACCTACA CCGAACTGAG ATACCTACAG CGTGAGCATT GAGAAAGCGC CACGTTCCC  
4681 GAAGGGAGAA AGGCGGACAG GTATCCGGTA AGCGGCAGGG TCGGAACAGG AGAGCGCAGC  
4741 AGGGAGCTTC CAGGGGGAAG CGCCTGGTAT CTTTATAGTC CTGTGCGGTT TCGCCACCTC  
4801 TGACTTGAGC GTCGATTTTT GTGATGCTCG TCAGGGGGGC GGAGCCTATG GAAAAACGCC  
4861 AGCAACGCGG CCTTTTACG GTTCTGGCC TTTGCTGGC CTTTTGCTCA CATGTTCTTT  
4921 CCTGCGTTAT CCCCTGATTC TGTGGATAAC CGTATTACCG CCTTTGAGTG AGCTGATACC  
4981 GCTCGCCGCA GCCGAACGAC CGAGCGCAGC GAGTCAGTGA GCGAGGAAGC GGAAGAGCGC  
5041 CTGATGCGGT ATTTTCTCCT TACGCATCTG TCGGGTATT CACACCGCAG ACCAGCCGCG  
5101 TAACCTGGCA AAATCGGTTA CGGTTGAGTA ATAAATGGAT GCCCTGCGTA AGCGGGTGTG  
5161 GGCGGACAAT AAAGTCTTAA ACTGAACAAA ATAGATCTAA ACTATGACAA TAAAGTCTTA  
5221 AACTAGACAG AATAGTTGTA AACTGAAATC AGTCCAGTTA TGCTGTGAAA AAGCATACTG  
5281 GACTTTTGTT ATGGCTAAAG CAAACTCTTC ATTTTCTGAA GTGCAAAATTG CCCGTCGTAT  
5341 TAAAGAGGGG CGTGGCCAAG GGCATGGTAA AGACTATATT CGCGGCGTTG TGACAATTTA  
5401 CCGAACAACCT CCGCGGCCGG GAAGCCGATC TCGGCTTGAA CGAATTGTTA GGTGGCGGTA  
5461 CTTGGGTCGA TATCAAAGTG CATCACTTCT TCCCGTATGC CCAACTTTGT ATAGAGAGCC  
5521 ACTGCGGGAT CGTCACCGTA ATCTGCTTGC ACGTAGATCA CATAAGCACC AAGCGCGTTG  
5581 GCCTCATGCT TGAGGAGATT GATGAGCGCG GTGGCAATGC CCTGCTCCCG GTGCTCGCCG  
5641 GAGACTGCGA GATCATAGAT ATAGATCTCA CTACGCGGCT GCTCAAACCT GGGCAGAACG  
5701 TAAGCCGCGA GAGCGCCAAC AACCGCTTCT TGGTCGAAGG CAGCAAGCGC GATGAATGTC  
5761 TTACTACGGA GCAAGTTCCC GAGGTAATCG GAGTCCGGCT GATGTTGGGA GTAGGTGGCT  
5821 ACGTCTCCGA ACTCACGACC GAAAAGATCA AGAGCAGCCC GCATGGATTT GACTTGGTCA  
5881 GGGCCGAGCC TACATGTGCG AATGATGCCC ATACTTGAGC CACCTAACTT TGTTTTAGGG  
5941 CGACTGCCCT GCTGCGTAAC ATCGTTGCTG CTGCGTAACA TCGTTGCTGC TCCATAACAT  
6001 CAAACATCGA CCCACGGCGT AACGCGCTTG CTGCTTGGAT GCCCGAGGCA TAGACTGTAC

62/240

6061 AAAAAACAG TCATAACAAG CCATGAAAAC CGCCACTGCG CCGTTACCAC CGCTGCGTTC  
6121 GGTC AAGGTT CTGGACCAGT TGGGTGAGCG CATACGCTAC TTGCATTACA GTTTACGAAC  
6181 CGAACAGGCT TATGTCAACT GGGTTCGTGC CTTTCATCCGT TTCCACGGTG TGCCTCACCC  
6241 GGCAACCTTG GGCAGCAGCG AAGTCGAGGC ATTTCTGTCC TGGCTGGCGA ACGAGCGCAA  
6301 GGTTTCGGTC TCCACGCATC GTCAGGCATT GGCGGCCTTG CTGTTCTTCT ACGGCAAGGT  
6361 GCTGTGCACG GATCTGCCCT GGCTTCAGGA GATCGGAAGA CCTCGGCCGT CGCGGCGCTT  
6421 GCCGGTGGTG CTGACCCCGG ATGAAGTGGT TCGCATCCTC GGTTTTCTGG AAGGCGAGCA  
6481 TCGTTTGTTT GCCCAGGACT CTAGCTATAG TTCTAGTGGT TGGCTA

FIGURE 28D



64/240

## pDEST9 12464 bp

<u>Location (Base Nos.)</u>	<u>Gene Encoded</u>
355..232	attR1
605..1264	CmR
1384..1468	inactivated ccdA
1606..1911	ccdB
1952..2078	attR2
2532..2782	ori
3482..4282	ampR
5232..5365	SP6 promoter
5365..6965	nsP1:non-structural protein 1
6965..9265	nsP2:non-structural protein 2
9265..10865	nsP3:non-structural protein 3
10865..161	nsP4:non-structural protein 4

1	AGCAAGTGGT	TCCGGACAGG	CTTGGGGGCC	GAAGTGGAGG	TGGCACTAAC	ATCTAGGTAT
61	GAGGTAGAGG	GCTGCAAAAG	TATCCTCATA	GCCATGGCCA	CCTTGGCGAG	GGACATTAAG
121	GC GTTTAAGA	AATTGAGAGG	ACCTGTTATA	CACCTCTACG	GCGGTCC TAG	ATTGGTGC GT
181	TAATACACAG	AATTCTGATT	GGATCCCGGT	CCGAAGCGCG	CTTTCCCATC	ACAAGTTTGT
241	ACAAAAAAGC	TGAACGAGAA	ACGTAAAATG	ATATAAATAT	CAATATATTA	AATTAGATTT
301	TGCATAAAAA	ACAGACTACA	TAATACTGTA	AAACACAACA	TATCCAGTCA	CTATGGCGGC
361	CGCTAAGTTG	GCAGCATCAC	CCGACGCACT	TTGCGCCGAA	TAAATACCTG	TGACGGAAGA
421	TCACTTCGCA	GAATAAATAA	ATCCTGGTGT	CCCTGTTGAT	ACCGGGAAGC	CCTGGGCCAA
481	CTTTTGGCGA	AAATGAGACG	TTGATCGGCA	CGTAAGAGGT	TCCAAC TTTC	ACCATAATGA
541	AATAAGATCA	CTACCGGGCG	TATTTTTTGA	GTTATCGAGA	TTTTCAGGAG	CTAAGGAAGC
601	TAAAATGGAG	AAAAAAATCA	CTGGATATAC	CACCGTTGAT	ATATCCCAAT	GGCATCGTAA
661	AGAACATTTT	GAGGCATTTT	AGTCAGTTGC	TCAATGTACC	TATAACCAGA	CCGTTCAGCT
721	GGATATTACG	GCCTTTTTTA	AGACCGTAAA	GAAAAATAAG	CACAAGTTT	ATCCGGCCTT
781	TATTCACATT	CTTGCCCGCC	TGATGAATGC	TCATCCGGAA	TTCCGTATGG	CAATGAAAGA
841	CGGTGAGCTG	GTGATATGGG	ATAGTGTTC	CCCTTGTTAC	ACCGTTTTC	ATGAGCAAAC
901	TGAAACGTTT	TCATCGCTCT	GGAGTGAATA	CCACGACGAT	TTCCGGCAGT	TTCTACACAT
961	ATATTCGCAA	GATGTGGCGT	GTTACGGTGA	AAACCTGGCC	TATTTCCCTA	AAGGGTTTAT
1021	TGAGAATATG	TTTTTCGTCT	CAGCCAATCC	CTGGGTGAGT	TTCACCA GTT	TTGATTTAAA
1081	CGTGGCCAAT	ATGGACA ACT	TCTTCGCCCC	CGTTTTTACC	ATGGGCAAAT	ATTATACGCA
1141	AGGCGACAAG	GTGCTGATGC	CGCTGGCGAT	TCAGGTTTCA	CATGCCGTCT	GTGATGGCTT
1201	CCATGTCGGC	AGAATGCTTA	ATGAATTACA	ACAGTACTGC	GATGAGTGGC	AGGGCGGGGC
1261	GTAAAGATCT	GGATCCGGCT	TACTAAAAGC	CAGATAACAG	TATGCGTATT	TGCGCGCTGA
1321	TTTTTGGCGT	ATAAGAATAT	ATACTGATAT	GTATACCCGA	AGTATGTCAA	AAAGAGGTGT
1381	GCTATGAAGC	AGCGTATTAC	AGTGACAGTT	GACAGCGACA	GCTATCAGTT	GCTCAAGGCA
1441	TATATGATGT	CAATATCTCC	GGTCTGGTAA	GCACAACCAT	GCAGAATGAA	GCCCGTCGTC
1501	TGCGTGCCGA	ACGCTGGAAA	GCGGAAAATC	AGGAAGGGAT	GGCTGAGGTC	GCCCGGTTTA
1561	TTGAAATGAA	CGGCTCTTTT	GCTGACGAGA	ACAGGGACTG	GTGAAATGCA	GTTTAAAGTT
1621	TACACCTATA	AAAGAGAGAG	CCGTTATCGT	CTGTTTGTGG	ATGTACAGAG	TGATATTATT
1681	GACACGCCCC	GGCGACGGAT	GGTGATCCCC	CTGGCCAGTG	CACGTCTGCT	GTGAGATAAA
1741	GTCTCCCGTG	AACTTTACCC	GGTGGTGCAT	ATCGGGGATG	AAAGCTGGCG	CATGATGACC
1801	ACCGATATGG	CCAGTGTGCC	GGTCTCCGTT	ATCGGGGAAG	AAGTGGCTGA	TCTCAGCCAC
1861	CGCGAAAATG	ACATCAAAAA	CGCCATTAAC	CTGATGTTCT	GGGGAATATA	AATGTCAGGC
1921	TCCCTTATAC	ACAGCCAGTC	TGCAGGTCGA	CCATAGTGAC	TGGATATGTT	GTGTTT TACA
1981	GTATTATGTA	GTCTGTTTTT	TATGCAAAAG	TGCTAATTTA	ATATATTGAT	ATTTATATCA
2041	TTTTACGTTT	CTCGTTCAGC	TTTCTTGTA	AAAGTGGTGA	TGGGAAC TCG	AGTTCACTAG
2101	TCGATCCCGC	GGCCGCTTTC	GAACTAGGC	AAGCATGCGG	GCCCAGTGGG	TAATTAATTG
2161	AATTACATCC	CTACGCAAAAC	GTTTTACGGC	CGCCGGTGCG	GCCCGCGCCC	GGCGGCCCGT
2221	CCTTGGCCGT	TGCAGGCCAC	TCCGGTGGCT	CCCGTCGTCC	CCGACTTCCA	GGCCAGCAG
2281	ATGCAGCAAC	TCATCAGCGC	CGTAAATGCG	CTGACAATGA	GACAGAACGC	AATTGCTCCT
2341	GCTAGGAGCT	TAATTGACG	AATAATTGGA	TTTTTATTTT	ATTTTGCAAT	TGGTTTTTAA
2401	TATTTCCAAA	AAAAAAAAAA	AAAAAAAAAA	AAAAAAAAAA	AAAAAAAAAA	AAAAAAAAAA

FIGURE 29B

2461 AAAAAAAAAA AAAAAAATA GAAATCGCGA TTTCTAGTCT GCATTAATGA ATCGGCCAAC  
 2521 GCGCGGGGAG AGGCGGTTTG CGTATTGGGC GCTCTTCCGC TTCCTCGCTC ACTGACTCGC  
 2581 TGCGCTCGGT CGTTCGGCTG CGGCGAGCGG TATCAGCTCA CTCAAAGGCG 3TAATACGGT  
 2641 TATCCACAGA ATCAGGGGAT AACGCAGGAA AGAACATGTG AGCAAAAGGC CAGCAAAAGG  
 2701 CCAGGAACCG TAAAAAGGCC GCGTTGCTGG CGTTTTTCCA TAGGCTCCGC CCCCTGACG  
 2761 AGCATCACAA AAATCGACGC TCAAGTCAGA GGTGGCGAAA CCGGACAGGA CTATAAAGAT  
 2821 ACCAGGCGTT TCCCCCTGGA AGCTCCCTCG TGCGCTCTCC TGTTCGACC CTGCCGCTTA  
 2881 CCGGATACCT GTCCGCCTTT CTCCCTTCGG GAAGCGTGGC GCTTTCTCAA TGCTCGCGCT  
 2941 GTAGGTATCT CAGTTCGGTG TAGGTCGTTT GCTCCAAGCT GGGCTGTGTG CACGAACCCC  
 3001 CCGTTCAGCC CGACCGCTGC GCCTTATCCG GTAACATATCG TCTTGAGTCC AACCCGGTAA  
 3061 GACACGACTT ATCGCCACTG GCAGCAGCCA CTGGTAACAG GATTAGCAGA GCGAGGTATG  
 3121 TAGGCGGTGC TACAGAGTTC TTGAAGTGGT GGCCTAACTA CGGCTACACT AGAAGGACAG  
 3181 TATTTGGTAT CTGCGCTCTG CTGAAGCCAG TTACCTTCGG AAAAAAGAGTT GGTAGCTCTT  
 3241 GATCCGGCAA ACAAAACCACC GCTGGTAGCG GTGGTTTTTT TGTGTGCAAG CAGCAGATTA  
 3301 CGCGCAGAAA AAAAGGATCT CAAGAAGATC CTTTGATCTT TTCTACGGGG TCTGACGCTC  
 3361 AGTGGAACGA AAATCAGCT TAAGGGATT TGGTCATGAG ATTATCAAAA AGGATCTTCA  
 3421 CCTAGATCCT TTTAAATTAA AAATGAAGTT TTAATCAAT CTAAAGTATA TATGAGTAAA  
 3481 CTTGGTCTGA CAGTTACCAA TGCTTAATCA GTGAGGCACC TATCTCAGCG ATCTGTCTAT  
 3541 TTCGTTTCAT CATAGTTGCC TGACTCCCGG TCGTGTAGAT AACTACGATA CGGGAGGGCT  
 3601 TACCATCTGG CCCCAGTGCT GCAATGATAC CGCGAGACCC ACGCTCACCG GCTCCAGATT  
 3661 TATCAGCAAT AAACCAGCCA GCCGGAAGGG CCGAGCGCAG AAGTGGTCCT GCAACTTTAT  
 3721 CCGCCTCCAT CCAGTCTATT AATTGTTGCC GGAAGCTAG AGTAAGTAGT TCGCCAGTTA  
 3781 ATAGTTTGCG CAACGTTGTT GCCATTGCTA CAGGCATCGT GGTGTACGC TCAGCTGTTG  
 3841 GTATGGCTTC ATTCAGCTCC GGTTCCTAAC GATCAAGGCG AGTTACATGA TCCCCCATGT  
 3901 TGTGCAAAAA AGCGGTTAGC TCCTTCGGTC CTCCGATCGT TGTGAGAAGT AAGTTGGCCG  
 3961 CAGTGTATAT ACTCATGGTT ATGGCAGCAC TGCATAATTC TCTTACTGTC ATGCCATCCG  
 4021 TAAGATGCTT TTCTGTGACT GGTGAGTACT CAACCAAGTC ATTCTGAGAA TAGTGTATGC  
 4081 GGCAGCCGAG TTGCTCTTGC CCGGCGTCAA TACGGGATAA TACCGCGCCA CATAGCAGAA  
 4141 CTTTAAAGT GCTCATCATT GAAAAACGTT CTTCCGGGCG AAAACTCTCA AGGATCTTAC  
 4201 CTGTGTTGAG ATCCAGTTG ATGTAACCCA CTCGTGCACC CAACTGATCT TCAGCATCTT  
 4261 TTACTTTTAC CAGCGTTTCT GGGTGAGCAA AAACAGGAAG GCAAAATGCC GCAAAAAAGG  
 4321 GAATAAGGGC GACACGAAA TGTGAATAC TCATACTCTT CCTTTTTCAA TATTATTGAA  
 4381 GCATTTATCA GGGTTATTGT CTCATGAGCG GATACATATT TGAATGTATT TAGAAAAATA  
 4441 AACAAATAGG GGTTCGCGC ACATTTCCCC GAAAAGTGCC ACCTGACGTC TAAGAAACCA  
 4501 TTATTATCAT GACATTAACC TATAAAAATA GGCGTATCAC GAGGCCCTTT CGTCTCGCGC  
 4561 GTTTCGGTGA TGACGGTGAA AACCTCTGAC ACATGCAGCT CCCGGAGACG GTCACAGCTT  
 4621 CTGTCTAAGC GGATGCCGGG AGCAGACAAG CCGCTCAGGG CCGCTCAGCG GGTGTTGGCG  
 4681 GGTGTGCGGG CTGGCTTAAC TATGCGGCAT CAGAGCAGAT TGTACTGAGA GTGCACCATA  
 4741 TCGACGCTCT CCCTTATGCG ACTCCTGCAT TAGGAAGCAG CCCAGTACTA GGTGAGGCC  
 4801 GTTGAGCACC GCCGCCGCAA GGAATGGTGC ATGCAAGGAG ATGGCGCCCA ACAGTCCCCC  
 4861 GGCCACGGGG CCTGCCACCA TACCCACGCC GAAACAAGCG CTCATGAGCC CGAAGTGGCG  
 4921 AGCCCGATCT TCCCCATCGG TGATGTCGGC GATATAGGCG CCAGCAACCG CACCTGTGGC  
 4981 GCCGGTGATG CCGGCCACGA TGCGTCCGGC GTAGAGGATC TGGCTAGCGA TGACCTGCT  
 5041 GATTGGTTG CTGACCATT TCCGGGTGCG GAACGGCGTT ACCAGAACT CAGAAGGTTT  
 5101 GTCCAACCAA ACCGACTCTG ACGGCAGTTT ACGAGAGAGA TGATAGGGTC TGCTTCAGTA  
 5161 AGCCAGATGC TACACAATTA GGCTTGATCA TATTGTCGTT AGAACGCGGC TACAATTAAT  
 5221 ACATAACCTT ATGTATCATA CACATACGAT TTAGGTGACA CTATAGATGG CGGATGTGTG  
 5281 ACATACACGA CGCCAAAAGA TTTTGTTCCT GCTCCTGCCA CCTCCGCTAC GCGAGAGATT  
 5341 AACCACCCAC GATGGCCGCC AAAGTGATG TTGATATTGA GGCTGACAGC CCATTATCA  
 5401 AGTCTTTGCA GAAGGCATTT CCGTCGTTG AGGTGGAGTC ATTGCAGGT ACACCAATG  
 5461 ACCATGCAAA TGCCAGAGCA TTTTCGCACC TGGCTACCAA ATTGATCGAG CAGGAGACTG  
 5521 ACAAAGACAC ACTCATCTTG GATATCGGCA GTGCGCCTTC CAGGAGAATG ATGTCTACGC  
 5581 ACAAATACCA CTGCGTATGC CCTATGCGCA GCGCAGAAGA CCCCAGAAAG CTCGATAGCT  
 5641 ACGCAAAGAA ACTGGCAGCG GCCTCCGGGA AGGTGCTGGA TAGAGAGATC GCAGGAAAAA  
 5701 TCACCGACCT GCAGACCGTC ATGGCTACG CAGACGCTGA ATCTCCTACC TTTTGCCTGC  
 5761 ATACAGACGT CACGTGTCGT ACGGCAGCCG AAGTGCCGT ATACCAGGAC GTGTATGCTG  
 5821 TACATGCACC AACATCGCTG TACCATCAGG CGATGAAAGG TGTCAGAACG GCGTATTGGA  
 5881 TTGGGTTTGA CACCACCCCG TTTATGTTTG ACGCGCTAGC AGGCGCGTAT CCAACCTACG-

FIGURE 29C

66/240

5941 CCACAAACTG GGCCGACGAG CAGGTGTTAC AGGCCAGGAA CATAGGACTG TGTGCA3CAT  
 6001 CCTTGACTGA GGAAGACTC GGCAAACTGT CCATTCTCCG CAAGAAGCAA TTGAAA3CTT  
 6061 GCGACACAGT CATGTTCTCG GTAGGATCTA CATTGTACAC TGAGAGCAGA AAGCTACTGA  
 6121 GGAGCTGGCA CTTACCCCTC GTATTCCACC TGAAAGGTAA ACAATCCTTT ACCTGTAGGT  
 6181 GCGATACCAT CGTATCATGT GAAGGGTACG TAGTTAAGAA AATCACTATG TGCCCC3GCC  
 6241 TGTACGGTAA AACGGTAGGG TACGCCGTGA CGTATCACGC GGAGGGATTG CTAGTG7GCA  
 6301 AGACCACAGA CACTGTCAAA GGAGAAAGAG TCTCATTCCC TGTATGCACC TACGTCC3CT  
 6361 CAACCATCTG TGATCAAATG ACTGGCATACT TAGCGACCGA CGTCACACCG GAGGAC3CAC  
 6421 AGAAGTTGTT AGTGGGATTG AATCAGAGGA TAGTTGTGAA CGGAAGAACA CAGCGAAACA  
 6481 CTAAACAGAT GAAGAACTAT CTGCTTCCGA TTGTGGCCGT CGCATTAGC AAGTGG3CGA  
 6541 GGGAAATACAA GGCAGACCTT GATGATGAAA AACCTCTGGG TGTCCGAGAG AGGTCACTTA  
 6601 CTTGCTGCTG CTTGTGGGCA TTTAAACGA GGAAGATGCA CACCATGTAC AAGAAACAG  
 6661 ACACCCAGAC AATAGTGAAG GTGCCTTCAG AGTTTAACTC GTTCGTCATC CCGAGC3TAT  
 6721 GGTCTACAGG CCTCGCAATC CCAGTCAGAT CACGCATTAA GATGCTTTTG GCCAAGAAGA  
 6781 CCAAGCGAGA GTTAATACCT GTTCTCGACG CGTCGTCAGC CAGGGATGCT GAACAA3AGG  
 6841 AGAAGGAGAG GTTGGAGGCC GAGCTGACTA GAGAAGCCTT ACCACCCCTC GTCCCCATCG  
 6901 CGCCGGCGGA GACGGGAGTC GTCGACGTCG ACGTTGAAGA ACTAGAGTAT CACGCA3GTG  
 6961 CAGGGGTCGT GGAAACACCT CGCAGCGCGT TGAAAGTCAC CGCACAGCCG AACGAC3TAC  
 7021 TACTAGGAAA TTACGTAGTT CTGTCCCGC AGACCGTGCT CAAGAGCTCC AAGTTG3CCC  
 7081 CCGTGCACCC TCTAGCAGAG CAGGTGAAAA TAATAACACA TAACGGGAGG GCCGGC3GTT  
 7141 ACCAGGTCGA CGGATATGAC GGCAGGGTCC TACTACCATG TGGATCGGCC ATTCCG3TCC  
 7201 CTGAGTTTCA GGCTTTGAGC GAGAGCGCCA CTATGGTGTA CAACGAAAGG GAGTTC3TCA  
 7261 ACAGGAAACT ATACCATATT GCCGTTTACG GACCCTCGCT GAACACCGAC GAGGAGA3AT  
 7321 ACGAGAAAGT CAGAGCTGAA AGAACTGACG CCGAGTACGT GTTCGACGTA GATAAAA3AT  
 7381 GCTGCGTCAA GAGAGAGGAA GCGTCGGGTT TGGTGTGTTG GGGAGAGCTA ACCAAC3CCC  
 7441 CGTTCATGA ATTGCGCTAC GAAGGGCTGA AGATCAGGCC GTCGGCACCA TATAAGACTA  
 7501 CAGTAGTAGG AGTCTTTGGG GTTCCGGGAT CAGGCAAGTC TGCTATTATT AAGAGC3TCG  
 7561 TGACCAAAACA CGATCTGGTC ACCAGCGGCA AGAAGGAGAA CTGCCAGGAA ATAGTTA3ACG  
 7621 ACGTGAAGAA GCACCGCGGG AAGGGGACAA GTAGGGA3AAA CAGTGACTCC ATCCTG3TAA  
 7681 ACGGGTGTG TCGTGCCGTG GACATCCTAT ATGTGGACGA GGCTTTTCGT TGCCATT3CCG  
 7741 GTACTCTGCT GGCCCTAATT GCTCTTGTTA AACCTCGGAG CAAAGTGGTG TTATGC3GAG  
 7801 ACCCAAGCA ATGCGGATTC TTCAATATGA TGCAGCTTAA GGTGAACCTC AACCACA3ACA  
 7861 TCTGCACTGA AGTATGTCAT AAAAGTATAT CCAGACGTTG CACGCGTCCA GTCACG3CCA  
 7921 TCGTGTCTAC GTTGCACTAC GGAGGCAAGA TGCGCACGAC CAACCCGTGC AACAA3CCA  
 7981 TAATCATAGA CACCACAGGA CAGACCAAGC CCAAGCCAGG AGACATCGTG TTAACAT3GCT  
 8041 TCCGAGGCTG GGCAAAGCAG CTGCAGTTGG ACTACCGTGG ACACGAAGTC ATGACA3CAG  
 8101 CAGCACTCA GGGCCTCACC CGCAAAGGGG TATACGCCGT AAGGCAGAAG GTGAAT3AAA  
 8161 ATCCCTTGTA TGCCCTGCG TCGGAGCAGC TGAATGTACT GCTGACGCGC ACTGAT3ATA  
 8221 GGCTGGTGTG GAAAACGCTG GCCGGCGATC CCTGGATTAA GGTCTATCA AACATT3CAC  
 8281 AGGGTAACTT TACGGCCACA TTGGAAGAAT GGCAAGAAGA ACACGACAAA ATAATGA3AGG  
 8341 TGATTGAAGG ACCGGCTGCG CCTGTGGACG CGTTCCAGAA CAAAGCGAAC GTGTGT3GGG  
 8401 CGAAAAGCCT GGTGCCTGTC CTGGACACTG CCGGAATCAG ATTGACAGCA GAGGAG3GGA  
 8461 GCACCATAAT TACAGCATTT AAGGAGGACA GAGCTTACTC TCCAGTGGTG GCCTTGA3ATG  
 8521 AAATTTGCAC CAAGTACTAT GGAGTTGACC TGGACAGTGG CCTGTTTTCT GCCCGA3AGG  
 8581 TGTCCCTGTA TTACGAGAAC AACCCTGGG ATAACAGACC TGGTGAAGG ATGTAT3GAT  
 8641 TCAATGCCGC AACAGCTGCC AGGCTGGAAG CTAGACATAC CTTCTGAAG GGGCAG3GGC  
 8701 ATACGGGCAA GCAGGCAGTT ATCGCAGAAA GAAAAATCCA ACCGCTTCT GTGCTG3ACA  
 8761 ATGTAATTCC TATCAACCGC AGGCTGCCGC ACGCCCTGGT GGCTGAGTAC AAGACG3TTA  
 8821 AAGGCAGTAG GGTTGAGTGG CTGGTCAATA AAGTAAGAGG GTACCACGTC CTGCTG3TGA  
 8881 GTGAGTACAA CCTGGCTTTG CCTCGACGCA GGGTCACTTG GTTGTACCG CTGAAT3TCA  
 8941 CAGGCGCCGA TAGGTGCTAC GACCTAAGTT TAGGACTGCC GGCTGACGCC GGCAGG3TCG  
 9001 ACTTGGTCTT TGTGAACATT CACACGGAAT TCAGAATCCA CCACTACCAG CAGTGT3TCG  
 9061 ACCACGCCAT GAAGCTGCAG ATGCTTGGGG GAGATGCGCT ACGACTGCTA AAACCC3GCG  
 9121 GCATCTTGAT GAGAGCTTAC GGATACGCCG ATAAAATCAG CGAAGCCGTT GTTCTCT3CT  
 9181 TAAGCAGAAA GTTCTCGTCT GCAAGAGTGT TGCGCCCGGA TTGTGTCACC AGCAAT3ACAG  
 9241 AAGTGTCTT GCTGTTCTCC AACTTTGACA ACGGAAAGAG ACCCTCTACG CTACAC3AGA  
 9301 TGAATACCAA GCTGAGTGCC GTGTATGCCG GAGAAGCCAT GCACACGGCC GGGTGT3CAC  
 9361 CATCTACAG AGTTAAGAGA GCAGACATAG CCACGTGCAC AGAAGCGGCT GTGGTT3ACG

FIGURE 29A



67/240

9421 CAGCTAACGC CCGTGGAAC TTAGGGGATG GCGTATGCAG GGCCGTGGCG AAGAAATGGC  
 9481 CGTCAGCCTT TAAGGGAGCA GCAACACCAG TGGGCACAAT TAAAACAGT ATGTGCGGCT  
 9541 CGTACCCCGT CATCCACGCT GTAGCGCCTA ATTTCTCTGC CACGACTGAA GCGGAAGGGG  
 9601 ACCGCGAATT GSCCGCTGTC TACCGGGCAG TGGCCGCCGA AGTAAACAGA CTGTCACTGA  
 9661 GCAGCGTAGC CATCCCGCTG CTGTCCACAG GAGTGTTTCAG CGGCGGAAGA GATAGGCTGC  
 9721 AGCAATCCCT CAACCATCTA TTCACAGCAA TGGACGCCAC GGACGCTGAC GTGACCATCT  
 9781 ACTGCAGAGA CAAAAGTTGG GAGAAGAAAA TCCAGGAAGC CATTGACATG AGGACGGCTG  
 9841 TGGAGTTGCT CAATGATGAC GTGGAGCTGA CCACAGACTT GGTGAGAGTG CACCCGGACA  
 9901 GCAGCCTGGT GGGTCGTAAG GGCTACAGTA CCACTGACGG GTCGCTGTAC TCGTACTTTG  
 9961 AAGGTACGAA ATTCAACCAG TCTGCTATTG ATATGGCAGA GATACTGACG TTGTGGCCCA  
 10021 GACTGCAAGA GGCAAACGAA CAGATATGCC TATACGCGCT GGGCGAAACA ATGGACAACA  
 10081 TCAGATCCAA ATGTCCGGTG AACGATTCCG ATTCATCAAC ACCTCCAGG ACAGTGCCCT  
 10141 GCCTGTGCCG CTACGCAATG ACAGCAGAAC GGATCGCCCG CCTTAGGTCA CACCAAGTTA  
 10201 AAAGCATGGT GTTTGCTCA TCTTTTCCCC TCCCGAAATA CCATGTAGAT GGGGTGCAGA  
 10261 AGGTAAAGTG CGAGAAGGTT CTCCTGTTTCG ACCCGACGGT ACCTTCAGTG GTTAGTCCGC  
 10321 GGAAGTATGC CGCATCTACG ACGGACCATT CAGATCGGTC GTTACGAGGG TTTGACTTTG  
 10381 ATGGGACCAC CGACTCGTCT TCCACTGCCA GCGATAACCAT GTCGCTACCC AGTTTGCAGT  
 10441 CGTGTGACAT CGACTCGATC TACGAGCCAA TGGCTCCCAT AGTAGTGACG GCTGACGTAC  
 10501 ACCCTGAACC CGCAGGCATC GCGGACCTGG CGGCAGATGT GCACCCTGAA CCCGCAGACC  
 10561 ATGTGGACCT GGAGAACCCG ATTCCTCCAC CGCGCCCGAA GAGAGCTGCA TACCTGCGCT  
 10621 CCCGCGCGCG GGAGCGACCG GTGCCGCGCG CGAGAAAGCC GACGCTGCC CCAAGGACTG  
 10681 CGTTTAGGAA CAAGCTGCCT TTGACGTTTCG GCGACTTTGA CGAGCACGAG GTCGATGCGT  
 10741 TGGCCTCCGG GATTACTTTC GGAGACTTCG ACGACGTCCT GCGACTAGGC CGCGCGGGTG  
 10801 CATATATTTT CTCCTCGGAC ACTGGCAGCG GACATTTACA ACAAAAATCC GTTAGGCAGC  
 10861 ACAATCTCCA GTGCGCACAA CTGGATGCGG TCCAGGAGGA GAAAATGTAC CCGCCAAAT  
 10921 TGGATACTGA GAGGGAGAAG CTGTGCTGTC TGAAAATGCA GATGCACCCA TCGGAGGCTA  
 10981 ATAAGAGTCG ATACCACTCT CGCAAAGTGG AGAACATGAA AGCCACGGTG GTGGACAGGC  
 11041 TCACATCGGG GGCCAGATTG TACACGGGAG CGGACGTAGG CCGCATACCA ACATACGCGG  
 11101 TTCGGTACCC CCGCCCCGTG TACTCCCCTA CCGTGATCGA AAGATTCTCA AGCCCCGATG  
 11161 TAGCAATCGC AGCGTGCAAC GAATACCTAT CCAGAAATTA CCCAACAGTG GCGTCGTACC  
 11221 AGATAACAGA TGAATACGAC GCATACTTGG ACATGGTTGA CGGGTCGGAT AGTTGCTTGG  
 11281 ACAGAGCGAC ATTCTGCCCC GCGAAGCTCC GGTGCTACCC GAAACATCAT GCGTACCACC  
 11341 AGCCGACTGT ACBCAGTGCC GTCCCGTCAC CTTTTCAGAA CACACTACAG AACGTGCTAG  
 11401 CGGCTGCCAC CAAGAGAAAC TGCAACGTCA CGCAAATGCG AGAACTACCC ACCATGGACT  
 11461 CGGCAGTGTT CAACGTGGAG TGCTTCAAGC GCTATGCCCTG CTCCGGAGAA TATTGGGAAG  
 11521 AATATGCTAA ACAACCTATC CGGATAACCA CTGAGAACAT CACTACCTAT GTGACCAAAT  
 11581 TGAAAGGCCC GAAAGCTGCT GCCTTGTTTCG CTAAGACCCA CAACTTGGTT CCGCTGCAGG  
 11641 AGGTTCCCAT GGACAGATTG ACGGTCGACA TGAAACGAGA TGTCAAAGTC ACTCCAGGGA  
 11701 CGAAACACAC AGAGGAAAGA CCCAAAGTCC AGGTAATTCA AGCAGCGGAG CCATTGGCGA  
 11761 CCGCTTACCT GTGCGGCATC CACAGGGAAT TAGTAAGGAG ACTAAATGCT GTGTTACGCC  
 11821 CTAACGTGCA CACATTGTTT GATATGTGCG CCGAAGACTT TGACGCGATC ATCGCCTCTC  
 11881 ACTTCCACCC AGGAGACCCG GTTCTAGAGA CGGACATTGC ATCATTCGAC AAAAGCCAGG  
 11941 ACGACTCCTT GGCTCTTACA GGTTTAATGA TCCTCGAAGA TCTAGGGGTG GATCAGTACC  
 12001 TGCTGGACTT GATCGAGGCA GCCTTTGGGG AAATATCCAG CTGTCACCTA CCAACTGGCA  
 12061 CGCGCTTCAA GTTCGGAGCT ATGATGAAAT CGGGCATGTT TCTGACTTTG TTTATTAACA  
 12121 CTGTTTTGAA CATCACCATA GCAAGCAGGG TACTGGAGCA GAGACTCACT GACTCCGCTT  
 12181 GTGCGGCCTT CATCGCGGAC GACAACATCG TTCACGGAGT GATCTCCGAC AAGCTGATGG  
 12241 CGGAGAGGTG CGCGTCGTGG GTCAACATGG AGGTGAAGAT CATTGACGCT GTCATGGGCG  
 12301 AAAAACCCCC ATATTTTTGT GGGGGATTGA TAGTTTTTGA CAGCGTCACA CAGACCGCTT  
 12361 GCCGTGTTTC AGACCCACTT AAGCGCCTGT TCAAGTTGGG TAAGCCGCTA ACAGCTGAAG  
 12421 ACAAGCAGGA CGAAGACAGG CGACGAGCAC TGAGTGACGA GGTT

FIGURE 29E

68/240

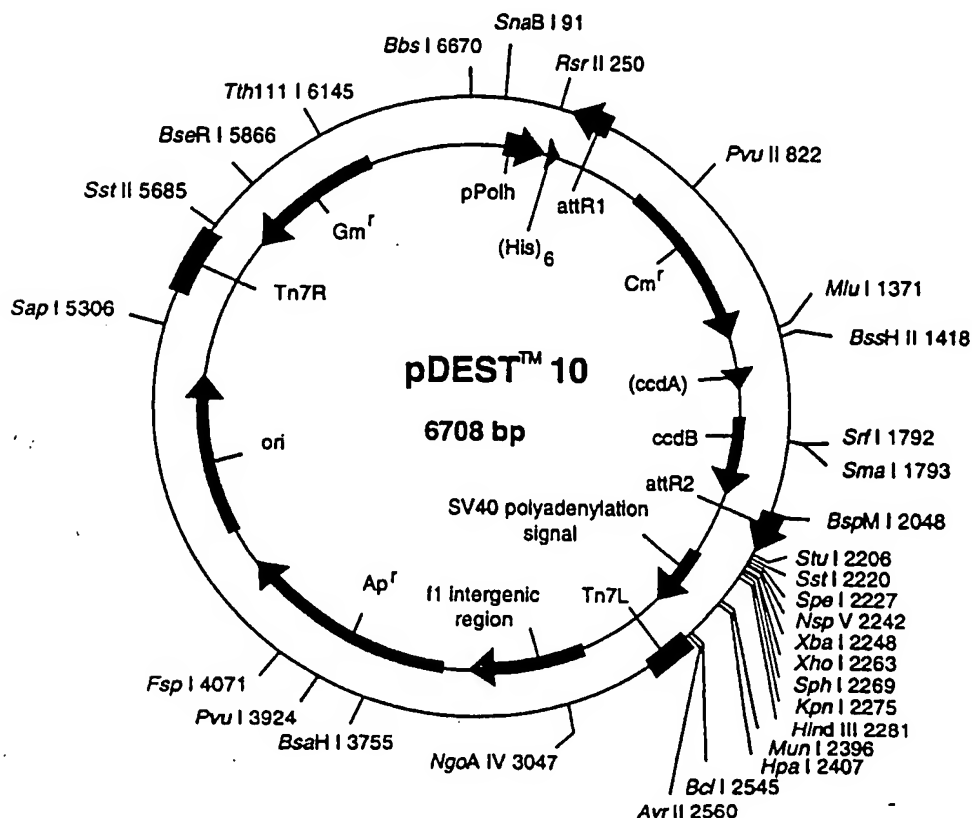
**Figure 30A: pDEST10 Polyhedron Promoter with N-His6, Baculovirus Transfer Plasmid**

154      <sup>→ mRNA from polyhedrin promoter</sup>  
aaa taa gta ttt tac tgt ttt cgt aac agt ttt gta ata aaa aaa cct ata  
ttt att cat aaa atg aca aaa gca ttg tca aaa cat tat ttt ttt gga tat

205  
aat att ccg gat tat tca tac cgt ccc acc atc ggg cgc gga tct cgg tcc  
tta taa ggc cta ata agt atg gca ggg tgg tag ccc gcg cct aga gcc agg

256      Met Ser Tyr Tyr His His His His His His Asp Tyr Asp Ile Pro  
gaa acc atg tcg tac tac cat cac cat cac cat cac cat gat tac gat atc cca  
ctt tgg tac agc atg atg gta gtg gta gtg gta gtg cta atg cta tag ggt

307      TEV protease  
Thr Thr Glu Asn Leu Tyr Phe Gln Gly Ile Thr Ser Leu Tyr Lys Lys  
acg acc gaa aac ctg tat ttt cag ggc atc ~~aca agt ttg tac aac aaa ggc~~  
tgc tgg ctt ttg gac ata aaa gtc ccg tag ~~tgt tca aac atg ttc ttc gga~~  
att R1 Int



69/240

## pDEST10 6708 bp

<u>Location (Base Nos.)</u>	<u>Gene Encoded</u>
23..152	Ppolh
461..337	attR1
711..1370	CmR
1490..1574	inactivated ccdA
1712..2017	ccdB
2058..2182	attR2
3394..4369	ampR
4510..5164	ori
5658..62	genR

```

1  CCCCCGATGA AGTGGTTCGC ATCCTCGGTT TTCTGGAAGG CGAGCATCGT TTGTTCCGCC
61 AGGACTCTAG CTATAGTTCT AGTGGTGGC TACGTATACT CCGGAATATT AATAGATCAT
121 GGAGATAATT AAAATGATAA CCATCTCGCA AATAAATAAG TATTTTACTG TTTTCGTAAC
181 AGTTTTGTAA TAAAAAACCC TATAAATATT CCGGATTATT CATACCGTCC CACCATCGGG
241 CGCGGATCTC GGTCCGAAAC CATGTCGTAC TACCATCACC ATCACCATCA CGATTACGAT
301 ATCCCAACGA CCGAAACCT GTATTTTCAG GGCATCACAA GTTTGTACAA AAAAGCTGAA
361 CGAGAAACGT AAAATGATAT AAATATCAAT ATATTAAATT AGATTTTGCA TAAAAACAG
421 ACTACATAAT ACTGTAAAC ACAACATATC CAGTCACTAT GGCGGCCGCT AAGTTGGCAG
481 CATCACCCGA CGCACTTTGC GCCGAATAAA TACCTGTGAC GGAAGATCAC TTCGCAGAAT
541 AAATAAATCC TGGTGTCCTT GTTGATACCG GGAAGCCCTG GGCCAACCTT TGGCGAAAAAT
601 GAGACGTTGA TCGGCACGTA AGAGGTTCCA ACTTTCACCA TAATGAAATA AGATCACTAC
661 TGGCGGTATT TTTGAGTTA TCGAGATTTT CAGGAGCTAA GGAAGCTAAA ATGGAGAAAA
721 AAATCACTGG ATATACCACC GTTGATATAT CCAATGGCA TCGTAAAGAA CATTTTGAGG
781 CATTTCACTC AGTTGCTCAA TGTACCTATA ACCAGACCGT TCAGCTGGAT ATTACGGCCT
841 TTTTAAAGAC CGTAAAGAAA AATAAGCACA AGTTTATCC GGCCTTTATT CACATTCTTG
901 CCCGCCGTGAT GAATGCTCAT CCGGAATCC GTATGGCAAT GAAAGACGGT GAGCTGGTGA
961 TATGGGATAG TGTTACCCCT GTTACACCG TTTTCCATGA GCAAACGTAA ACGTTTTCAT
1021 CGCTCTGGAG TGAATACCAC GACGATTTC GGCAGTTTCT ACACATATAT TCGCAAGATG
1081 TGGCGGTGTTA CGGTGAAAC CTGGCCTATT TCCCTAAAGG GTTTATTGAG AATATGTTTT
1141 TCGTCTCAGC CAATCCCTGG GTGAGTTTCA CCAGTTTGA TTTAAACGTG GCCAATATGG
1201 ACAACTTCTT CGCCCCCGTT TTCACCATGG GCAAATATTA TACGCAAGGC GACAAGGTGC
1261 TGATGCCGCT GCGGATTCAG GTTCATCATG CCGTCTGTGA TGGCTTCCAT GTCGGCAGAA
1321 TGCTTAATGA ATTACAACAG TACTGCGATG AGTGGCAGGG CGGGGCGTAA ACGCGTGGAT
1381 CCGGCTTACT AAAAGCCAGA TAACAGTATG CGTATTTGCG CGCTGATTTT TGCGGTATAA
1441 GAATATATAC TGATATGTAT ACCCGAAGTA TGCAAAAAG AGGTGTGCTA TGAAGCAGCG
1501 TATTACAGTG ACAGTTGACA GCGACAGCTA TCAGTTGCTC AAGGCATATA TGATGTCAAT
1561 ATCTCCGGTC TGGTAAGCAC AACCATGCAG AATGAAGCCC GTCGTCTCGG TGCCGAACGC
1621 TGGAAAGCGG AAAATCAGGA AGGGATGGCT GAGGTCGCCC GGTTTATTGA AATGAACGGC
1681 TCTTTTGCTG ACGAGAACAG GGAAGTGTGA AATGCAGTTT AAGGTTTACA CCTATAAAG
1741 AGAGAGCCGT TATCGTCTGT TTGTGGATGT ACAGAGTGAT ATTATTGACA CGCCCGGGCG
1801 ACGGATGGTG ATCCCCCTGG CCAGTGCACG TCTGCTGTCA GATAAAGTCT CCCGTGAACT
1861 TTACCCGGTG GTGCATATCG GGGATGAAAG CTGGCGCATG ATGACCACCG ATATGGCCAG
1921 TGTGCCGGTC TCCGTTATCG GGAAGAAGT GGCTGATCTC AGCCACCGCG AAAATGACAT
1981 CAAAAACGCC ATTAACCTGA TGTTCGGGG AATATAAATG TCAGGCTCCC TTATACACAG
2041 CCAGTCTGCA GGTGACCAT AGTGACTGGA TATGTTGTGT TTTACAGTAT TATGTAGTCT
2101 GTTTTATATG CAAAATCTAA TTTAATATAT TGATATTTAT ATCATTTTAC GTTCTCGTCT
2161 CAGCTTTCTT GTACAAAGTG GTGATGCCAT GGATCCGGAA TTCAAAGGCC TACGTCGACG
2221 AGCTCAACTA GTGCGGCCGC TTTCGAATCT AGAGCCTGCA GTCTCGAGGC ATGCGGTACC
2281 AAGCTTGTCG AGAAGTACTA GAGGATCATA ATCAGCCATA CCACATTGTG AGAGGTTTAA
2341 CTTGCTTTAA AAAACCTCCC ACACCTCCCC CTGAACCTGA AACATAAAAT GAATGCAATT
2401 GTTGTGTGTTA ACTTGTTTAT TGCAGCTTAT AATGGTTACA AATAAAGCAA TAGCATCACA
2461 AATTTACAAA ATAAAGCATT TTTTCACTG CATTCTAGTT GTGGTTTGTC CAAACTCATC
2521 AATGTATCTT ATCATGTCTG GATCTGATCA CTGCTTGAGC CTAGGAGATC CGAACCAGAT
2581 AAGTGAAATC TAGTTCACAA CTATTTGTGC ATTTTAAAT TTCGTATTAG CTTACGACGC-

```

FIGURE 30B

70/240

2641 TACACCCAGT TCCCATCTAT TTTGTCACTC TTCCCTAAAT AATCCTTAAA AACTCCATTT  
2701 CCACCCCTCC CAGTTCCCAA CTATTTTGTC CGCCACAGC GGGGCATTTT TCTTCCTGTT  
2761 ATGTTTTTAA TCAAACATCC TGCCAACTCC ATGTGACAAA CCGTCATCTT CGGCTACTTT  
2821 TTCTCTGTCA CAGAATGAAA ATTTTCTGT CATCTCTTCG TTATTAATGT TTGTAATTGA  
2881 CTGAATATCA ACGCTTATTT GCAGCCTGAA TGGCGAATGG GACGCGCCCT GTAGCGCGC  
2941 ATTAAGCGCG GCGGGTGTGG TGGTTACGCG CAGCGTGACC GCTACACTTG CCAGCGCCCT  
3001 AGCGCCCGCT CCTTCGCTT TCTTCCCTTC CTTTCTCGCC ACGTTGCGCG GCTTTCCCG  
3061 TCAAGCTCTA AATCGGGGGC TCCCTTTAGG GTTCCGATTT AGTGCTTTAC GGCACCTCGA  
3121 CCCCCAAAAA CTTGATTAGG GTGATGGTTC ACGTAGTGGG CCATCGCCCT GATAGACGGT  
3181 TTTTCGCCCT TTGACGTTGG AGTCCACGTT CTTTAATAGT GGACTCTTGT TCCAACTGG  
3241 AACCAACTC AACCCATCT CGGTCTATTC TTTTGATTTA TAAGGGATT TGC CGATTTC  
3301 GGCCTATTGG TTAATAAATG AGCTGATTTA AAAAAATTT AACGCGAATT TTAACAAAAT  
3361 ATTAACGTTT ACAATTTTCA GTGGCACTTT TCGGGGAAAT GTGCGCGGAA CCCCTATTTG  
3421 TTTATTTTTC TAAATACATT CAAATATGTA TCCGCTCATG AGACAATAAC CCTGATAAAT  
3481 GCTTCAATAA TATTGAAAAA GGAAGAGTAT GAGTATTCAA CATTTCCGTG TCGCCCTTAT  
3541 TCCCTTTTTT GCGGCATTTT GCCTTCCTGT TTTTGCTCAC CCAGAAACGC TGGTGAAAGT  
3601 AAAAGATGCT GAAGATCAGT TGGGTGCACG AGTGGGTAC ATCGAACTGG ATCTCAACAG  
3661 CCGTAAGATC CTTGAGAGTT TTCGCCCCGA AGAACGTTTT CCAATGATGA GCACCTTTAA  
3721 AGTTCGTCTA TGTGGCGCGG TATTATCCCG TATTGACGCC GGGCAAGAGC AACTCGGTG  
3781 CCGCATACAC TATTCTCAGA ATGACTTGGT TGAGTACTCA CCAGTCACAG AAAAGCATCT  
3841 TACGGATGGC ATGACAGTAA GAGAATTATG CAGTGCTGCC ATAACCATGA GTGATAACAC  
3901 TGCGGCCAAC TTACTTCTGA CAACGATCGG AGGACCGAAG GAGCTAACCG CTTTTTTGCA  
3961 CAACATGGGG GATCATGTAA CTCGCCTTGA TCGTTGGGAA CCGGAGCTGA ATGAAGCCAT  
4021 ACCAAACGAC GAGCGTGACA CCACGATGCC TGTAGCAATG GCAACAACGT TGC GCAAACT  
4081 ATTAACCTGC GAACTACTTA CTCTAGCTTC CCGGCAACAA TTAATAGACT GGATGGAGGC  
4141 GGATAAAGTT GCAGGACCAC TTCTGCGCTC GGCCCTTCG GCTGGCTGGT TTATGTGTA  
4201 TAAATCTGGA GCCGGTGAGC GTGGGTCTCG CGGTATCATT GCAGCACTGG GGCCAGATGG  
4261 TAAGCCCTCC CGTATCGTAG TTATCTACAC GACGGGGAGT CAGGCAACTA TGGATGAACG  
4321 AAATAGACAG ATCGCTGAGA TAGGTGCCCTC ACTGATTAAG CATTGGTAAC TGTGACACCA  
4381 AGTTTACTCA TATATACTTT AGATTGATTT AAAACTTCAT TTTTAATTTA AAAGGATCTA  
4441 GGTGAAGATC CTTTTTGATA ATCTCATGAC CAAAATCCCT TAACGTGAGT TTTCTGTCCA  
4501 CTGAGCGTCA GACCCCGTAG AAAAGATCAA AGGATCTTCT TGAGATCCTT TTTTCTGCG  
4561 CGTAATCTGC TGCTTGCAAA CAAAAAACC ACCGCTACCA GCGGTGGTTT GTTGCCGGA  
4621 TCAAGAGCTA CCAACTCTTT TTCCGAAGGT AACTGGCTTC AGCAGAGCGC AGATACCAAA  
4681 TACTGTCTTT CTAGTGTAGC CGTAGTTAGG CCACCCTTC AAGAACTCTG TAGCACCGCC  
4741 TACATACCTC GCTCTGCTAA TCCTGTTACC AGTGGCTGCT GCCAGTGGCG ATAAGTCGTG  
4801 TCTTACCGGG TTGGACTCAA GACGATAGTT ACCGGATAAG GCGCAGCGGT CCGGCTGAAC  
4861 GGGGGGTTCG TGCACACAGC CCAGCTTGA GCGAACGACC TACACCGAAC TGAGATACCT  
4921 ACAGCGTGAG CATTGAGAAA GCGCCACGCT TCCCGAAGGG AGAAAGGCGG ACAGGTATCC  
4981 GGTAAAGCGG AGGGTCGGAA CAGGAGAGCG CACGAGGGAG CTTCCAGGGG GAAACGCTG  
5041 GTATCTTTAT AGTCTGTGCG GGTTCGCCA CCTCTGACTT GAGCGTCGAT TTTGTGATG  
5101 CTCGTGAGG GGGCGGAGCC TATGAAAAA CGCCAGCAAC GCGGCCTTTT TACGGTTCCT  
5161 GGCTTTTTCG TGGCCTTTTG CTCACATGTT CTTTCTGCG TTATCCCCTG ATTCTGTGGA  
5221 TAACCGTATT ACCGCCTTTG AGTGAGCTGA TACCGCTCGC CGCAGCCGAA CGACCGAGCG  
5281 CAGCGAGTCA GTGAGCGAGG AAGCGGAAGA GCGCCTGATG CGGTATTTTC TCCTTACGCA  
5341 TCTGTGCGGT ATTTACACC GCAGACCAGC CGCGTAACCT GGCAAAATCG GTTACGGTTG  
5401 AGTAATAAAT GGATGCCCTG CGTAAGCGGG TGTGGGCGGA CAATAAAGTC TTAACCTGAA  
5461 CAAAATAGAT CTAAACTATG ACAATAAAGT CTTAAACTAG ACAGAATAGT TGTAAACTGA  
5521 AATCAGTCCA GTTATGCTGT GAAAAAGCAT ACTGGACTTT TGTATGGCT AAAGCAAAC  
5581 CTTCAATTTT TGAAGTCAA ATTGCCCGTC GTATTAAAGA GGGGCGTGGC CAAGGGCATG  
5641 GTAAAGACTA TATTCGCGG GTTGTGACAA TTTACCGAAC AACTCCGCGG CCGGGAAGCC  
5701 GATCTCGGCT TGAACGAATT GTTAGGTGGC GGTACTTGGG TCGATATCAA AGTGCATCAC  
5761 TTCTTCCCGT ATGCCCAACT TTGTATAGAG AGCCACTGCG GGATCGTCAC CGTAATCTGC  
5821 TTGCACGTAG ATCACATAAG CACCAAGCGC GTTGGCCTCA TGCTTGAGGA GATTGATGAG  
5881 CGCGGTGGCA ATGCCCTGCC TCCGGTGCTC GCCGGAGACT GCGAGATCAT AGATATAGAT  
5941 CTCCTACGCG GGCTGCTCAA ACCTGGGCG AACGTAAGCC GCGAGAGCGC CAACAACCGC  
6001 TTCTTGGTGC AAGGCAGCAA GCGCGATGAA TGTCTTACTA CCGAGCAAGT TCCCGAGGTA  
6061 ATCGGAGTCC GGCTGATGTT GGGAGTAGGT GGCTACGTCT CCGAACTCAC GACCGAAAAG-

Figure 30C

6121 ATCAAGAGCA GCCCGCATGG ATTTGACTTG GTCAGGGCCG AGCCTACATG TGCGAATGAT  
6181 GCCCATACTT GAGCCACCTA ACTTTGTTTT AGGGCGACTG CCCTGCTGCG TAACATCGTT  
6241 GCTGCTGCGT AACATCGTTG CTGCTCCATA ACATCAAACA TCGACCCACG GCGTAACGCG  
6301 CTTGCTGCTT GGATGCCCCG GGCATAGACT GTACAAAAAA ACAGTCATAA CAAGCCATGA  
6361 AAACCGCCAC TGCGCCGTTA CCACCGCTGC GTTCGGTCAA GGTTCTGGAC CAGTTGCGTG  
6421 AGCGCATACG TACTTGCAAT TACAGTTTAC GAACCGAACA GGCTTATGTC AACTGGGTTC  
6481 GTGCCTTCAT CCGTTTCCAC GGTGTGCGTC ACCCGGCAAC CTTGGGCAGC AGCGAAGTCG  
6541 AGGCATTTCT GTCCTGGCTG GCGAACGAGC GCAAGGTTTC GGTCTCCACG CATCGTCAGG  
6601 CATTGGCGGC CTGCTGTTC TTCTACGGCA AGGTGCTGTG CACGGATCTG CCCTGGCTTC  
6661 AGGAGATCGG AAGACCTCGG CCGTCGCGG GCTTGCCGGT GGTGCTGA

72/240

Figure 31A:

pDEST11

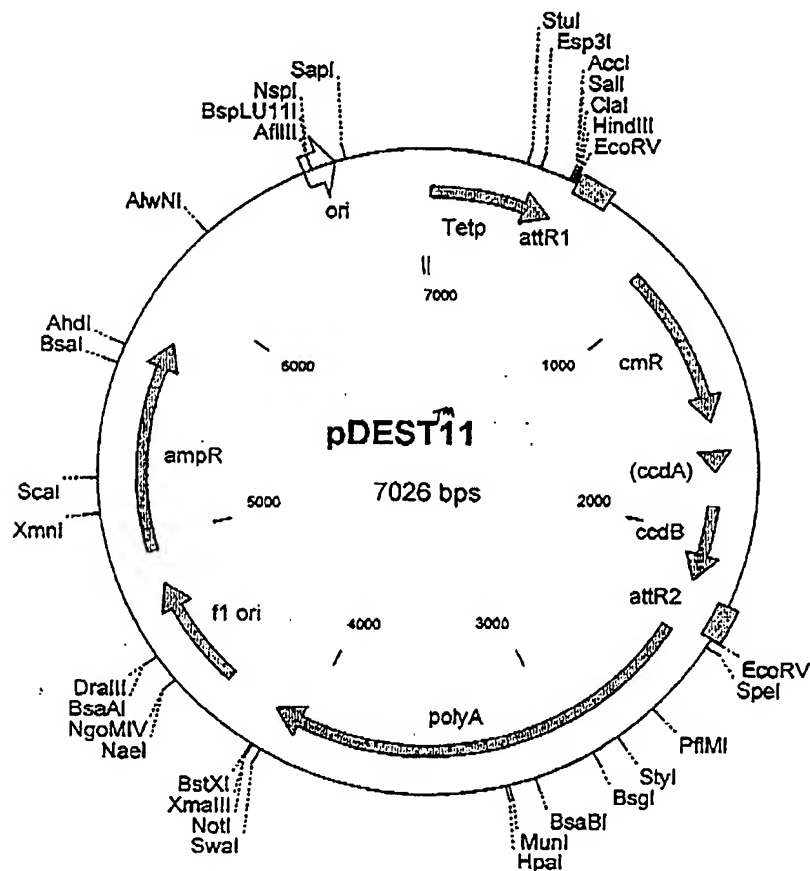
**Tet-regulated eukaryotic expression**

358 tag tga acc ~~gfc~~ <sup>mRNA from CMV promoter (controlled by tetracycline)</sup> aga tcg cct gga gac gcc atc cac gct gtt ttg acc tcc  
 atc act tgg cag tct agc gga cct ctg cgg tag gtg cga caa aac tgg agg

409 ata gaa gac acc ggg acc gat cca gcc tcc gcg gcc ccg aat tcg agc tcg  
 tat ctt ctg tgg ccc tgg cta ggt cgg agg cgc cgg ggc tta agc tcg agc

460 gta ccc ggg gat cct cta gag tcg agg <sup>Sal</sup> <sup>Cla</sup> <sup>Hind3</sup> <sup>EcoRV</sup> ~~tcg acg gta tcg ata lagc ttg ata~~  
 cat ggg ccc cta gga gat ctc agc tcc agc ~~ggc cat agc tat tcg aac tat~~

511 tca ~~aca agt ttg~~ <sup>Tet</sup> ~~aa~~ <sup>attR1</sup> ~~aaa gct gaa cga gaa acg taa~~ ~~aat gat ata gat~~  
 agt ~~tgt tca aac atg ttt~~ ~~tct cga ctt gct ctc tgc att tta cta tat tta~~



73/240

## pDEST11 7026 bp

<u>Location (Base Nos.)</u>	<u>Gene Encoded</u>
4..479	Tetp ((Tet operator) 7 and min hCMV promoter)
638..514	attR1
888..1547	CmR
1667..1751	inactivated ccdA
1889..2194	ccdB
2235..2359	attR2
2402..4132	polyA
4347..4803	f1 ori
4940..5797	ampR

```

1 CGAGTTTACC ACTCCCTATC AGTGATAGAG AAAAGTGAAA GTCGAGTTTA CCACTCCCTA
61 TCAGTGATAG AGAAAAGTGA AAGTCGAGTT TACCACTCCC TATCAGTGAT AGAGAAAAGT
121 GAAAGTCGAG TTTACCACTC CCTATCAGTG ATAGAGAAAA GTGAAAGTCG AGTTTACCAC
181 TCCCTATCAG TGATAGAGAA AAGTGAAAGT CGAGTTTACC ACTCCCTATC AGTGATAGAG
241 AAAAGTGAAA GTCGAGTTTA CCACTCCCTA TCAGTGATAG AGAAAAGTGA AAGTCGAGCT
301 CGGTACCCGG GTCGAGTAGG CGTGTACGGT GGGAGGCCTA TATAAGCAGA GCTCGTTTAG
361 TGAACCGTCA GATCGCCTGG AGACGCCATC CACGCTGTTT TGACCTCCAT AGAAGACACC
421 GGGACCGATC CAGCCTCCGC GGCCCCGAAT TCGAGCTCGG TACCCGGGGA TCCTCTAGAG
481 TCGAGGTCGA CGGTATCGAT AAGCTTGATA TCAACAAGTT TGTACAAAAA AGCTGAACGA
541 GAAACGTAAA ATGATATAAA TATCAATATA TTAAATTAGA TTTTGCATAA AAAACAGACT
601 ACATAATACT GTAAACACA ACATATCCAG TCACTATGGC GGCCGCTAAG TTGGCAGCAT
661 CACCCGACGC ACTTTGCGCC GAATAAATAC CTGTGACGGA AGATCACTTC GCAGAATAAA
721 TAAATCCTGG TGTCCTGTT GATACCGGGA AGCCCTGGGC CAACTTTTGG CGAAAATGAG
781 ACGTTGATCG GCACGTAAGA GGTTCCAACT TTCACCATAA TGAAATAAGA TCACTACCGG
841 GCGTATTTT TGAGTTATCG AGATTTTCAG GAGCTAAGGA AGCTAAAATG GAGAAAAAAA
901 TCACTGGATA TACCACCGTT GATATATCCC AATGGCATCG TAAAGAACAT TTTGAGGCAT
961 TTCAGTCAGT TGCTCAATGT ACCTATAACC AGACCGTTCA GCTGGATATT ACGGCCTTTT
1021 TAAAGACCGT AAAGAAAAAT AAGCACAAGT TTTATCCGGC CTTTATTAC ATTCTTGCCC
1081 GCCTGATGAA TGCTCATCCG GAATTCGGTA TGGCAATGAA AGACGGTGAT CTGGTGATAT
1141 GGGATAGTGT TCACCCTTGT TACACCGTTT TCCATGAGCA AACTGAAACG TTTTCATCGC
1201 TCTGGAGTGA ATACCACGAC GATTTCCGGC AGTTTCTACA CATATATTCG CAAGATGTGG
1261 CGTGTTACGG TGAACACCTG GCCTATTTCC CTAAAGGGTT TATTGAGAAT ATGTTTTTCG
1321 TCTCAGCCAA TCCCTGGGTG AGTTTCACCA GTTTTGATTT AAACGTGGCC AATATGGACA
1381 ACTTCTTCGC CCCCGTTTTT ACCATGGGCA AATATTATAC GCAAGGCGAC AAGGTGCTGA
1441 TGCCGCTGGC GATTCAGGTT CATCATGCCG TCTGTGATGG CTTCCATGTC GGCAGAATGC
1501 TTAATGAATT ACAACAGTAC TGCGATGAGT GGCAGGCGCG GCGGTAAAGA TCTGGATCCG
1561 GCTTACTAAA AGCCAGATAA CAGTATGCGT ATTTGCGCGC TGATTTTTGC GGTATAAGAA
1621 TATATACTGA TATGTATACC CGAAGTATGT CAAAAAGAGG TGTGCTATGA AGCAGCGTAT
1681 TACAGTGACA GTTGACAGCG ACAGCTATCA GTTGCTCAAG GCATATATGA TGTCAATATC
1741 TCCGGTCTGG TAAGCACAAC CATGCAGAAT GAAGCCCGTC GTCTGCGTGC CGAACGCTGG
1801 AAAGCGGAAA ATCAGGAAGG GATGGCTGAG GTCGCCCGGT TTATTGAAAT GAACGGCTCT
1861 TTTGCTGACG AGAACAGGGA CTGGTGAAAT GCAGTTTAAG GTTTACACCT ATAAAAGAGA
1921 GAGCCGTTAT CGTCTGTTG TGGATGTACA GAGTGATATT ATTGACACGC CCGGGCGACG
1981 GATGGTGATC CCCCTGGCCA GTGCACGTCT GCTGTGAGAT AAAGTCTCCC GTGAACTTTA
2041 CCCGGTGGTG CATATCGGGG ATGAAAGCTG GCGCATGATG ACCACCGATA TGGCCAGTGT
2101 GCCGGTCTCC GTTATCGGGG AAGAAGTGGC TGATCTCAGC CACCGCGAAA ATGACATCAA
2161 AAACGCCATT AACCTGATGT TCTGGGGAAT ATAAATGTCA GGCTCCCTTA TACACAGCCA
2221 GTCTGCAGGT CGACCATAGT GACTGGATAT GTTGTGTTTT ACAGTATTAT GTAGTCTGTT
2281 TTTTATGCAA AATCTAATTT AATATATTGA TATTTATATC ATTTTACGTT TCTCGTTTCA
2341 CTTTCTTGTA CAAAGTGTTT GATATCGAAT TCCTGCAGCC CGGGGGATCT ATAGTTCTTA
2401 GAGCACTCGC ATGAGTGGCA GGGCGGGGCG TAATTTTTTT AAGGCAGTTA TTGGTGCCCT
2461 TAAACGCCTG GTGCTACGCC TGAATAAGTG ATAATAAGCG GATGAATGGC AGAAATTTCG
2521 CGGATCTTTG TGAAGGAACC TTACTTCTGT GGTGTGACAT AATTGGACAA ACTACCTACA-

```

FIGURE 31B

2581 GAGATTTAAA GCTCTAAGGT AAATATAAAA TTTTAAAGTG TATAATGTGT TAAACTACTG  
2641 ATTCTAATTG TTTGTGTATT TTAGATTCCA ACCTATGGAA CTGATGAATG GGAGCAGTGG  
2701 TGGAAATGCCT TTAATGAGGA AAACCTGTTT TGCTCAGAAG AAATGCCATC TAGTGATGAT  
2761 GAGGCTACTG CTGACTCTCA ACATTCTACT CCTCCAAAAA AGAAGAGAAA GGTAGAAGAC  
2821 CCAAGGACT TTCCTTCAGA ATTGCTAAGT TTTTGTAGTC ATGCTGTGTT TAGTAATAGA  
2881 ACTCTTGCTT GCTTTGCTAT TTACACCACA AAGGAAAAAG CTGCACTGCT ATACAAGAAA  
2941 ATTATGGAAG AATATTCTGT AACCTTTATA AGTAGGCATA ACAGTTATAA TCATAACATA  
3001 CTGTTTTTTC TTAATCCACA CAGGCATAGA GTGTCTGCTA TTAATAACTA TGCTCAAAAA  
3061 TTGTGTACCT TTAGCTTTTT AATTTGTAAA GGGGTTAATA AGGAATATTT GATGTATAGT  
3121 GCCTTGACTA GAGATCATAA TCAGCCATAC CACATTTGTA GAGGTTTTAC TTGCTTTAAA  
3181 AACCTCCCA CACCTCCCCC TGAACCTGAA ACATAAAATG AATGCAATTG TTGTTGTTAA  
3241 CTTGTTTTATT GCAGCTTATA ATGGTTACAA ATAAAGCAAT AGCATCACAA ATTTACACAA  
3301 TAAAGCATTT TTTTCACTGC ATTCTAGTTG TGGTTTGTCC AAACCTCATCA ATGTATCTTA  
3361 TCATGTCTGG ATCCCCAGGA AGCTCCTCTG TGTCTCATA AACCTAACC TCCTCTACTT  
3421 GAGAGGACAT TCCAATCATA GGCTGCCCAT CCACCCTCTG TGTCTCTCTG TTAATTAGGT  
3481 CACTTAACAA AAAGGAAATT GGGTAGGGGT TTTTCACAGA CCGCTTTCTA AGGGTAATTT  
3541 TAAAATATCT GGAAGTCCC TTCCACTGCT GTGTTCCAGA AGTGTGGTA AACAGCCCAC  
3601 AAATGTCAAC AGCAGAAACA TACAAGCTGT CAGCTTTGCA CAAGGGCCCA ACACCTGCT  
3661 CATCAAGAAG CACTGTGGTT GCTGTGTTAG TAATGTGCAA AACAGGAGGC ACATTTTCCC  
3721 CACCTGTGTA GGTTCACAAA TATCTAGTGT TTTTATTTT ACTTGATCA GGAACCCAGC  
3781 ACTCCACTGG ATAAGCATTA TCCTTATCCA AAACAGCCTT GTGGTCAGTG TTCATCTGCT  
3841 GACTGTCAAC TGTAGCATTT TTTGGGGTTA CAGTTTGAGC AGGATATTTG GTCCTGTAGT  
3901 TTGCTAACAC ACCCTGCAGC TCCAAAGGTT CCCACCAAC AGCAAAAAAA TGAAAATTTG  
3961 ACCCTTGAAT GGGTTTTCCA GCACCATTTT CATGAGTTTT TTGTGTCCCT GAATGCAAGT  
4021 TTAACATAGC AGTTACCCCA ATAACCTCAG TTTTAACAGT AACAGCTTCC CACATCAAAA  
4081 TATTTCCACA GGTAAAGTCC TCATTTAAAT TAGGCAAAGG AATTGCTCTA GAGCGGCCGC  
4141 CACCGCGGTG GAGCTCCAAT TCGCCCTATA GTGAGTCGTA TTACGCGCGC TCACTGGCCG  
4201 TCGTTTTACA ACGTCGTGAC TGGGAAAACC CTGGCGTTAC CCAACTTAAT CGCCTTGCAG  
4261 CACATCCCCC TTTCCGCCAGC TGGCGTAATA GCGAAGAGGC CCGCACCAGT CGCCCTTCCC  
4321 AACAGTTGCG CAGCCTGAAT GGCGAATGGG ACGCGCCCTG TAGCGGCGCA TTAAGCGCGG  
4381 CGGGTGTGGT GGTTACGCGC AGCGTGACCG CTACACTTGC CAGCGCCCTA GCGCCCGCTC  
4441 CTTTCGCTTT CTTCCCTTCC TTTCTCGCCA CGTTCGCCGG CTTTCCCGT CAAGCTCTAA  
4501 ATCGGGGGCT CCCTTTAGGG TTCCGATTTA GTGCTTTACG GCACCTCGAC CCAAAAAAAC  
4561 TTGATTAGGG TGATGGTTCA CGTAGTGGGC CATCGCCCTG ATAGACGGTT TTTGCGCCTT  
4621 TGACGTTGGA GTCCACGTTT TTTAATAGTG GACTCTTGTT CCAAACTGGA ACAACACTCA  
4681 ACCCTATCTC GGTCTATTCT TTTGATTAT AAGGGATTTT GCCGATTTG GCCTATTGGT  
4741 TAAAAAATGA GCTGATTAA CAAAAATTTA ACGCGAATTT TAACAAAATA TTAACGCTTA  
4801 CAATTTAGGT GGCATTTTTC GGGGAAATGT GCGCGGAACC CCTATTTGTT TATTTTTCTA  
4861 AATACATTCA AATATGTATC CGCTCATGAG ACAATAACCC TGATAAATG TTCAATAATA  
4921 TTGAAAAAGG AAGAGTATGA GTATTCAACA TTTCCGTGTC GCCCTTATTC CTTTTTTGC  
4981 GGCATTTTGC CTTCTGTGTT TTGCTCACCC AGAAACGCTG GTGAAAAGTAA AAGATGCTGA  
5041 AGATCAGTTG GGTGCACGAG TGGGTTACAT CGAACTGGAT CTCAACAGCG GTAAGATCCT  
5101 TGAGAGTTTT CGCCCGAAG AACGTTTTCC AATGATGAGC ACTTTTAAAG TTCTGCTATG  
5161 TGGCGCGGTA TTATCCCGTA TTGACGCCGG GCAAGAGCAA CTCGGTCCGC GCATACACTA  
5221 TTCTCAGAA GACTTGTTG AGTACTCACC AGTCACAGAA AAGCATCTTA CGGATGGCAT  
5281 GACAGTAAGA GAATTATGCA GTGCTGCCAT AACCATGAGT GATAACACTG CGGCCAACTT  
5341 ACTTCTGACA ACGATCGGAG GACCGAAGGA GCTAACCGCT TTTTGCACA ACATGGGGGA  
5401 TCATGTAAC CGCCTTGATC GTTGGGAACC GGAGCTGAAT GAAGCCATAC CAAACGACGA  
5461 GCGTGACACC ACGATGCCTG TAGCAATGGC AACAACGTTG CGCAAACTAT TAACTGGCGA  
5521 ACTACTTACT CTAGCTTCCC GGCAACAATT AATAGACTGG ATGGAGGCGG ATAAAGTTGC  
5581 AGGACCACTT CTGCGCTCGG CCCTTCCGGC TGGCTGTTTT ATTGCTGATA AATCTGGAGC  
5641 CGGTGAGCGT GGGTCTCGCG GTATCATTGC AGCACTGGGG CCAGATGGTA AGCCCTCCCG  
5701 TATCGTAGTT ATCTACAGA CGGGGAGTCA GGCAACTATG GATGAACGAA ATAGACAGAT  
5761 CGCTGAGATA GGTGCCTCAC TGATTAGCA TTGGTAACTG TCAGACCAAG TTTACTCATA  
5821 TATACTTTAG ATTGATTAA AACTTCATTT TTAATTTAAA AGGATCTAGG TGAAGATCCT  
5881 TTTTGATAAT CTCATGACCA AAATCCCTTA ACGTGAGTTT TCGTTCCACT GAGCGTCAGA  
5941 CCCCCTAGAA AAGATCAAAG GATCTTCTTG AGATCCTTTT TTTCTGCGCG TAATCTGCTG  
6001 CTTGCAAACA AAAAAACCAC CGTACCAGC GGTGGTTTGT TTGCCGGATC AAGAGCTACC-

FIGURE 31C



75/240

6061 AACTCTTTTT CCGAAGGTAA CTGGCTTCAG CAGAGCGCAG ATACCAAATA CTGTCCTTCT  
6121 AGTGTAGCEG TAGTTAGGCC ACCACTTCAA GAACTCTGTA GCACCGCCTA CATACCTCGC  
6181 TCTGCTAATC CTGTTACCAG TGGCTGCTGC CAGTGGCGAT AAGTCGTGTC TTACCGGGTT  
6241 GGA CTCAAGA CGATAGTTAC CGGATAAGGC GCAGCGGTCG GGCTGAACGG GGGGTTTCGTG  
6301 CACACAGCCC AGCTTGGAGC GAACGACCTA CACCGAACTG AGATACCTAC AGCGTGAGCT  
6361 ATGAGAAAGC GCCACGCTTC CCGAAGGGAG AAAGGCGGAC AGGTATCCGG TAAGCGGCAG  
6421 GGTCCGAACA GGAGAGCGCA CGAGGGAGCT TCCAGGGGGA AACGCCTGGT ATCTTTATAG  
6481 TCCTGTCGGG TTTGCCACC TCTGACTTGA GCGTCGATT TTGTGATGCT CGTCAGGGGG  
6541 GCGGAGCCTA TGGAAAAACG CCAGCAACGC GGCCTTTTAA CGGTTCTGG CCTTTTGCTG  
6601 GCCTTTTGCT CACATGTTCT TTCCTGCGTT ATCCCCTGAT TCTGTGGATA ACCGTATTAC  
6661 CGCCTTTGAG TGAGCTGATA CCGCTCGCCG CAGCCGAACG ACCGAGCGCA GCGAGTCAGT  
6721 GAGCGAGGAA GCGGAAGAGC GCCCAATACG CAAACCGCCT CTCCCCGCGC GTTGCCCGAT  
6781 TCATTAATGC AGCTGGCACG ACAGGTTTCC CGACTGGAAA GCGGGCAGTG AGCGCAACGC  
6841 AATTAATGTG AGTTAGCTCA CTCATTAGGC ACCCCAGGCT TTACACTTTA TGCTTCCGGC  
6901 TCGTATGTTG TGTGGAATTG TGAGCGGATA ACAATTTAC ACAGGAAACA GCTATGACCA  
6961 TGATTACGCC AAGCGCGCAA TTAACCCTCA CTAAAGGGAA CAAAAGCTGG GTACCGGGCC  
7021 CCCCCT

FIGURE 31D

76/240

**Figure 32A: pDEST12.2 CMV Promoter for Eukaryotic Expression, SV40 Promoter/ori for G418 Resistance**

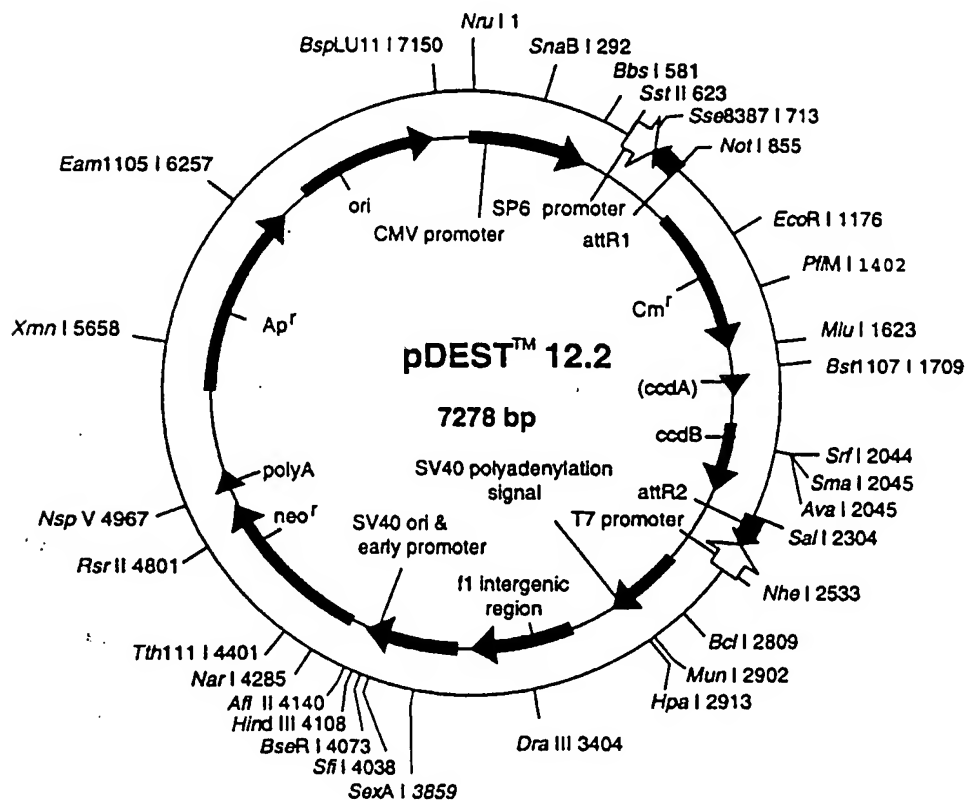
307 *← mRNA from CMV promoter*  
 acc gtc aga tcg cct gga gac gcc atc cac gct gtt ttg acc tcc ata gaa  
 tgg cag tct agc gga cct ctg cgg tag gtg cga caa aac tgg agg tat ctt

358 gac acc ggg acc gat cca gcc tcc gga ctc tag cct agg ccg cgg agc gga  
 ctg tgg ccc tgg cta ggt cgg agg cct gag atc gga tcc ggc gcc tcg cct

409 taa caa ttt cac aca gga aac agc tat gac cat tag gcc ttt gca aaa agc  
 att gtt aaa gtg tgt cct ttg tcg ata ctg gta atc cgg aaa cgt ttt tcg

460 tat tta ggt gac act ata gaa ggt acg cct gca ggt *Age* *EcoRI*  
 ata aat cca ctg tga tat ctt cca tgc gga cgt cca tgg cca ggc ctt aag  
*Int* *attR1*

511 cca tca ~~aca agt ttg taa ada ada gct gaa cga gaa acg taa aat gat ata~~  
 ggt agt ~~tgt tca aac atg ttt ttt cga ctt gct ctt tgc att sta cta tat~~



77/240

## pDEST12.2 7278 bp (rotated to position 3900)

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
86..136		ori
220..742		CMV promoter
1059..935		attR1
1168..1827		CmR
1947..2031		inactivated ccdA
2169..2474		ccdB
2515..2639		attR2
2824..3186		small t & polyA
3310..3378		lac
4363..5157		neo
5680..6540		ampR

1	GGGGGGCGGA	GCCTATGGAA	AAACGCCAGC	AACGCGGCCT	TTTTACGGTT	CCTGGCCTTT
61	TGCTGGCCTT	TTGCTCACAT	GTTCTTTCCT	GCGTTATCCC	CTGATTCTGT	GGATAACCGT
121	ATTACCGCCT	TTGAGTGAGC	TGATACCGCT	CGCCGCAGCC	GAACGACCGA	GCGCAGCGAG
181	TCAGTGAGCG	AGGAAGCGGA	AGAGCTCGCG	AATGCATGTC	GTTACATAAC	TTACGGTAAA
241	TGGCCCGCCT	GGCTGACCGC	CCAACGACCC	CCGCCCATTG	ACGTCAATAA	TGACGTATGT
301	TCCCATAGTA	ACGCCAATAG	GGACTTTCCA	TTGACGTCAA	TGGGTGGAGT	ATTTACGGTA
361	AACTGCCCAC	TTGGCAGTAC	ATCAAGTGTA	TCATATGCCA	AGTACGCCCC	CTATTGACGT
421	CAATGACGGT	AAATGGCCCC	CCTGGCATT	TGCCCAGTAC	ATGACCTTAT	GGGACTTTCC
481	TACTTGGCAG	TACATCTACG	TATTAGTCAT	CGCTATTACC	ATGGTGATGC	GGTTTTGGCA
541	GTACATCAAT	GGGCGTGGAT	AGCGGTTTGA	CTCACGGGGA	TTTCCAAGTC	TCCACCCCAT
601	TGACGTCAAT	GGGAGTTTGT	TTTGGCACCA	AAATCAACGG	GACTTTCCAA	AATGTCGTAA
661	CAACTCCGCC	CCATTGACGC	AAATGGGCGG	TAGGCGTGTA	CGGTGGGAGG	TCTATATAAG
721	CAGAGCTCGT	TTAGTGAACC	GTCAGATCGC	CTGGAGACGC	CATCCACGCT	GTTTTGACCT
781	CAGATAGAGA	CACCGGGACC	GATCCAGCCT	CCGGACTCTA	GCCTAGGCCG	CGGGACGGAT
841	AACAATTTC	CACAGGAAAC	AGCTATGACC	ATTAGGCCTT	TGCAAAAAGC	TATTTAGGTG
901	ACACTATAGA	AGGTACGCCT	GCAGGTACCG	GATCACAAGT	TTGTACAAAA	AAGCTGAACG
961	AGAAACGTAA	AATGATATAA	ATATCAATAT	ATTAAATTAG	ATTTTGCATA	AAAAACAGAC
1021	TACATAATAC	TGTAAACAC	AACATATCCA	GTCATATGG	CGGCCGCATT	AGGCACCCCA
1081	GGCTTTACAC	TTTATGCTTC	CGGCTCGTAT	AATGTGTGGA	TTTTGAGTTA	GGATCCGTCG
1141	AGATTTTCAG	GAGCTAAGGA	AGCTAAAATG	GAGAAAAAAA	TCACTGGATA	TACCACCGTT
1201	GATATATCCC	AATGGCATCG	TAAAGAACAT	TTTGAGGCAT	TTCAAGTCAGT	TGCTCAATGT
1261	ACCTATAACC	AGACCGTTCA	GCTGGATATT	ACGGCCTTTT	TAAAGACCGT	AAAGAAAAAT
1321	AAGCACAAAGT	TTTATCCGGC	CTTTATTTCAC	ATTCTTGCCC	GCCTGATGAA	TGCTCATCCG
1381	GAATTCGGTA	TGGCAATGAA	AGACGGTGAG	CTGGTGATAT	GGGATAGTGT	TCACCCTTGT
1441	TACACCGTTT	TCCATGAGCA	AACTGAAACG	TTTTTCATCGC	TCTGGAGTGA	ATACCACGAC
1501	GATTTCCGGC	AGTTTCTACA	CATATATTCG	CAAGATGTGG	CGTGTTACGG	TGAAAACCTG
1561	GCCTATTTCC	CTAAAGGGTT	TATTGAGAAT	ATGTTTTTCG	TCTCAGCCAA	TCCCTGGGTG
1621	AGTTTCACCA	GTTTTGATTT	AAACGTGGCC	AATATGGACA	ACTTCTTCGC	CCCCGTTTTT
1681	ACCATGGGCA	AATATTATAC	GCAAGGCGAC	AAGGTGCTGA	TGCCGCTGGC	GATTCAGGTT
1741	CATCATGCCG	TCTGTGATGG	CTTCCATGTC	GGCAGAATGC	TTAATGAATT	ACAACAGTAC
1801	TGCGATGAGT	GGCAGGGCGG	GGCGTAAACG	CGTGGATCCG	GCTTACTAAA	AGCCAGATAA
1861	CAGTATGCGT	ATTTGCGCGC	TGATTTTTTC	GGTATAAGAA	TATATACTGA	TATGTATACC
1921	CGAAGTATGT	CAAAAAGAGG	TGTGCTATGA	AGCAGCGTAT	TACAGTGACA	GTTGACAGCG
1981	ACAGCTATCA	GTTGCTCAAG	GCATATATGA	TGTCAATATC	TCCGGTCTGG	TAAGCACAAAC
2041	CATGCAGAA	GAAGCCCGTC	GTCTGCGTGC	CGAACGCTGG	AAAGCGGAAG	ATCAGGAAGG
2101	GATGGCTGAG	GTCGCCCCGT	TTATTGAAAT	GAACGGCTCT	TTTGCTGACG	AGAACAGGGA
2161	CTGGTGAAAT	GCAGTTTAA	GTTTACACCT	ATAAAAGAGA	GAGCCGTTAT	CGTCTGTTTG
2221	TGGATGTACA	GAGTGATATT	ATTGACACGC	CCGGGCGACG	GATGGTGATC	CCCCTGGCCA
2281	GTGCACGTCT	GCTGTGAGAT	AAAGTCTCCC	GTGAACCTTA	CCCGGTGGTG	CATATCGGGG
2341	ATGAAAGCTG	GCGCATGATG	ACCACCGATA	TGGCCAGTGT	GCCGGTCTCC	GTTATCGGGG
2401	AAGAAGTGGC	TGATCTCAGC	CACCGCGAAA	ATGACATCAA	AAACGCCATT	AACCTGATGT-

FIGURE 32B

78/240

2461 TCTGGGGAAT ATAAATGTCA GGCTCCCTTA TACACAGCCA GTCTGCAGGT CGACCATAGT  
2521 GACTGGATAT GTTGTGTTTT ACAGTATTAT GTAGTCTGTT TTTTATGCAA AATCTAATTT  
2581 AATATATTGA TATTTATATC ATTTTACGTT TCTCGTTCAG CTTTCTTGTA CAAAGTGGTG  
2641 ATCGCGTGCA TCGCAGCTCA TAGCTCTCTC CCTATAGTGA GTCGTATTAT AAGCTAGGCA  
2701 CTGGCCGTCG TTTTACAACG TCGTGA CTGCTG GAAAAC TGCT AGCTTGGGAT CTTTGTGAAG  
2761 GAACCTTACT TCTGTGGTGT GACATAATTG GACAAACTAC CTACAGAGAT TTAAAGCTCT  
2821 AAGGTAAATA TAAAATTTTT AAGTGTATAA TGTGTTAAAC TAGCTGCATA TGCTTGCTGC  
2881 TTGAGAGTTT TGCTTACTGA GTATGATTTA TGAAAATATT ATACACAGGA GCTAGTGATT  
2941 CTAATTGTTT GTGTATTTTA GATTCACAGT CCCAAGGCTC ATTTACAGGCC CCTCAGTCCCT  
3001 CACAGTCTGT TCATGATCAT AATCAGCCAT ACCACATTTG TAGAGGTTT ACTTGCTTTA  
3061 AAAAACCTCC CACACCTCCC CCTGAACCTG AAACATAAAA TGAATGCAAT TGTGTTGTT  
3121 AACTTGTTTA TTGCAGCTTA TAATGGTTAC AAATAAAGCA ATAGCATCAC AAATTTACACA  
3181 AATAAAGCAT TTTTTCCTACT GCATTCTAGT TGTGGTTTGT CCAAACCTCAT CAATGTATCT  
3241 TATCATGTCT GGATCGATCC TGCAATTAATG AATCGGCCAA CGCGCGGGGA GAGCGGGTTT  
3301 GCGTATTGGC TGGCGTAATA GCGAAGAGGC CCGCACCGAT CGCCCTTCCC AACAGTTGCG  
3361 CAGCCTGAAT GGCGAATGGG ACGCGCCCTG TAGCGGCGCA TTAAGCGCGG CGGGTGTGGT  
3421 GAGTTACGCG ACAGTGACCG CTACACTTGC CAGCGCCCTA GCGCCCGCTC CTTTGCTTTT  
3481 CTTCCCTTCC TTTCTCGCCA CGTTCGCGG CTTTCCCGT CAAGCTCTAA ATCGGGGGCT  
3541 CCCTTTAGGG TTCCGATTTA GTGCTTTACG GCACCTCGAC CCCAAAAAAC TTGATTAGGG  
3601 TGATGGTTCA CGTAGTGGGC CATCGCCCTG ATAGACGGTT TTTCCGCCCT TGACGTTGGA  
3661 GTCCACGTTT TTTAATAGTG GACTCTTGT CCAAACTGGA ACAACACTCA ACCCTATCTC  
3721 GGTCTATTCT TTTGATTTAT AAGGGATTTT GCCGATTTCT GCCTATTGGT TAAAAAATGA  
3781 GCTGATTTAA CAAATATTTA ACGCGAATTT TAACAAAATA TTAACGTTTA CAATTTCCGC  
3841 TGATGCGGTA TTTCTCCTT ACGCATCTGT CCGGTATTTT ACACCGCAT CGCGGATCTG  
3901 CGCAGCACCA TGGCCTGAAA TAACCTCTGA AAGAGGAACT TGGTTAGGTA CCTTCTGAGG  
3961 CGGAAAGAAC CAGCTGTGGA ATGTGTGTCA GTTAGGGTGT GGAAAGTCCC CAGGCTCCCC  
4021 AGCAGGCAGA AGTATGCAAA GCATGCATCT CAATTAGTCA GCAACCAGGT GTGGAAAGTC  
4081 CCCAGGCTCC CCAGCAGGCA GAAGTATGCA AAGCATGCAT CTCAATTAGT CAGCAACCAT  
4141 AGTCCCGCCC CTAACCTCCG CCATCCCGCC CTAACCTCCG CCCAGTTCCG CCCATTCTCC  
4201 GCCCCATGGC TGAATAATTT TTTTATTTA TGCAGAGGCC GAGGCCGCTC CGGCTCTGA  
4261 GCTATTCCAG AAGTAGTGAG GAGGCTTTT TGGAGGCCTA GGCTTTTGCA AAAAGCTTGA  
4321 TTCTTCTGAC ACAACAGTCT CGAACTTAA GCTAGAGCCA CCATGATTGA ACAAGATGGA  
4381 TTGCACGCAG GTTCTCCGGC CGCTTGGGTG GAGAGGCTAT TCGGCTATGA CTGGGCACAA  
4441 CAGACAATCG GCTGCTCTGA TGCCGCGGTG TTCCGGCTGT CAGCGCAGGG GCGCCCGGTT  
4501 CTTTGTGTC AGACCGACCT GTCCGGTGCC CTGAATGAAC TGCAGGACGA GGCAGCGCGG  
4561 CTATCGTGGC TGGCCACGAC GGGCGTTTCT TGCGCAGCTG TGCTCGACGT TGCTACTGAA  
4621 GCGGGAAGGG ACTGGCTGCT ATTGGGCGAA GTGCCGGGGC AGGATCTCCT GTCATCTCAC  
4681 CTTTGCTCTG CCGAGAAAGT ATCCATCAT GCTGATGCAA TGCGGCGGCT GCATACGCTT  
4741 GATCCGGCTA CCTGCCCAT CGACCACAA GCGAAACAT GCATCGAGCG AGCAGCTACT  
4801 CGGATGGAAG CCGGTCTTGT CGATCAGGAT GATCTGGACG AAGAGCATCA GGGGCTCGCG  
4861 CCAGCCGAAC TGTTCCGCCAG GCTCAAGGCG CGCATGCCCG ACGGCGAGGA TCTCGTCGTG  
4921 ACCCATGGCG ATGCCTGCTT GCCGAATATC ATGGTGAAA ATGGCCGCTT TTCTGGATTCT  
4981 ATCGACTGTG GCCGGCTGGG TGTGGCGGAC CGCTATCAGG ACATAGCGTT GGCTACCCGT  
5041 GATATTGCTG AAGAGCTTGG CGGCGAATGG GCTGACCGCT TCCTCGTGCT TTACGGTATC  
5101 GCCGCTCCCG ATTCGCAGCG CATCGCCTTC TATCGCCTT TTGACGAGTT CTTCTGAGCG  
5161 GGA CTCTGGG GTTCGAAATG ACCGACCAAG CGACGCCCAA CCTGCCATCA CGATGGCCCG  
5221 AATAAAATAT CTTTATTTT ATTACATCTG TGTGTTGGTT TTTTGTGTGA ATCGATAGCG  
5281 ATAAGGATCC GCGTATGGTG CACTCTCAGT ACAATCTGCT CTGATGCCGC ATAGTTAAGC  
5341 CAGCCCCGAC ACCCGCCAAC ACCCGCTGAC GCGCCCTGAC GGGCTTGTCT GCTCCCGGCA  
5401 TCCGCTTACA GACAAGCTGT GACCGTCTCC GGGAGCTGCA TGTGTCAGAG GTTTTACCCG  
5461 TCATCACC GAACGCGCGAG ACGAAAGGGC CTCGTGATAC GCCTATTTT ATAGGTTAAT  
5521 GTCATGATAA TAATGGTTT TTAGACGTCA GGTGGCACTT TTCGGGAAA TGTGCGCGGA  
5581 ACCCTATTAT GTTTATTTT CTAAATACAT TCAAATATGT ATCCGCTCAT GAGACAATAA  
5641 CCCTGATAAA TGCTTCAATA ATATTGAAAA AGGAAGAGTA TGAGTATTCA ACATTTCCGT  
5701 GTCGCCCTTA TTCCCTTTT TGCGGCATTT TGCCCTTCTG TTTTGTCTCA CCCAGAAACG  
5761 CTGGTGAAAG TAAAAGATGC TGAAGATCAG TTGGGTGCAC GAGTGGGTTA CATCGAACTG  
5821 GATCTCAACA GCGGTAAGAT CCTTGAGAGT TTTCCGCCCG AAGAACGTTT TCCAATGATG  
5881 AGCACTTTTA AAGTTCTGCT ATGTGGCGCG GTATTATCCC GTATTGACGC CGGCAAGAG-

FIGURE 32C

79/240

5941 CAACTCGGTC GCCGCATACA CTATTCTCAG AATGACTTGG TTGAGTACTC ACCAGTCACA  
6001 GAAAAGCATC TTACGGATGG CATGACAGTA AGAGAATTAT GCAGTGCTGC CATAACCATG  
6061 AGTGATAACA CTGCGGCCAA CTTACTTCTG ACAACGATCG GAGGACCGAA GGAGCTAACC  
6121 GCTTTTTTGC ACAACATGGG GGATCATGTA ACTCGCCTTG ATCGTTGGGA ACCGGAGCTG  
6181 AATGAAGCCA TACCAAACGA CGAGCGTGAC ACCACGATGC CTGTAGCAA GGCAACAACG  
6241 TTGCGCAAAC TATTAAC TGG CGAACTACTT ACTCTAGCTT CCCGGCAACA ATTAATAGAC  
6301 TGGATGGAGG CGGATAAAGT TGCAGGACCA CTTCTGCGCT CGGCCCTTCC GGCTGGCTGG  
6361 TTTATTGCTG ATAAATCTGG AGCCGGTGAG CGTGGGTCTC GCGGTATCAT TGCAGCACTG  
6421 GGGCCAGATG GTAAGCCCTC CCGTATCGTA GTTATCTACA CGACGGGGAG TCAGGCAACT  
6481 ATGGATGAAC GAAATAGACA GATCGCTGAG ATAGGTGCCT CACTGATTAA GCATTGGTAA  
6541 CTGTCAGACC AAGTTTACTC ATATATACTT TAGATTGATT TAAAACCTCA TTTTAAATTT  
6601 AAAAGGATCT AGGTGAAGAT CCTTTTGTAT AATCTCATGA CCAAATCCC TTAACGTGAG  
6661 TTTTCGTTC ACTGAGCGTC AGACCCCGTA GAAAAGATCA AAGGATCTTC TTGAGATCCT  
6721 TTTTTTCTGC GCGTAATCTG CTGCTTGCAA ACAAAAAAAC CACCGCTACC AGCGGTGGTT  
6781 TGTTTGCCGG ATCAAGAGCT ACCAACTCTT TTTCCGAAGG TAACTGGCTT CAGCAGAGCG  
6841 CAGATACCAA ATACTGTCCT TCTAGTGTAG CCGTAGTTAG GCCACCACT CAAGAACTCT  
6901 GTAGCACCGC CTACATACCT CGCTCTGCTA ATCCTGTTAC CAGTGGCTGC TGCCAGTGGC  
6961 GATAAGTCGT GTCTTACCGG GTTGGACTCA AGACGATAGT TACCGGATAA GGCGCAGCGG  
7021 TCGGGCTGAA CGGGGGGFTC GTGCACACAG CCCAGCTTGG AGCGAACGAC CTACACCGAA  
7081 CTGAGATACC TACAGCGTGA GCATTGAGAA AGCGCCACGC TTCCCGAAGG GAGAAAGGCG  
7141 GACAGGTATC CGGTAAGCGG CAGGGTCGGA ACAGGAGAGC GCACGAGGGA GCTTCCAGGG  
7201 GGAAACGCCT GGTATCTTTA TAGTCCTGTC GGGTTTCGCC ACCTCTGACT TGAGCGTCTGA  
7261 TTTTTGTGAT GCTCGTCA

FIGURE 32D

80/240

Figure 33A:

pDEST13

Native protein in E. coli:  $\lambda$ PL  
promoter

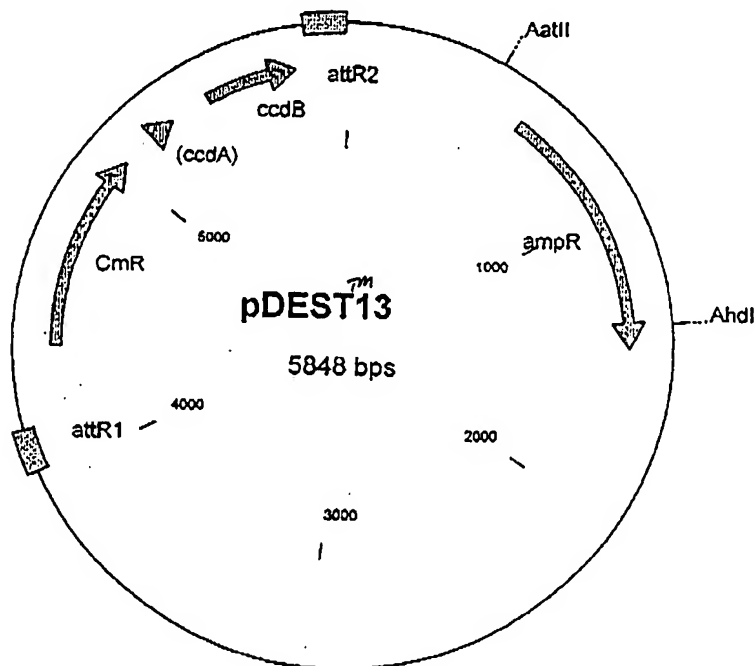
3721 tgggcaaacc aagacagcta aagatctctc acctaccaa caatgcccc ctgcaaaaa  
 acccgtttgg ttctgtcgat ttctagagag tggatggttt gttacggggg gacgtttttt

3781 taaattcata taaaaaacat acagataacc atctgcggtg ataaattatc tctggcgggtg  
 atttaagtat attttttgta tgtctattgg tagacgccac tatttaatag agaccgccac

3841 ttgacataaa taccactggc ggtgatactg agcacatcag caggacgcac tgaccaccat  
aactgtattt atggtgaccg ccactatgac tctgtagtc gtctgcggtg actggtggta

3901 gaaggtgacg ctcttaaaaa ttaagecctg aagaaggcca gcattcaaag cagaaggctt  
 cttccactgc gagaattttt aattcgggac tcttcccggt cgtaagtttc gtcttccgaa

3961 tgggggtgtgt gatacgaaac gaagcattgg gatcatcaca agtttgtaca aaaaagctga  
 accccacaca ctatgctttg cttcgttaacc ctagtatgt tcaaacatgt ttgttcgact



81/240

## pDEST13 5848 bp

<u>Location (Base Nos.)</u>	<u>Gene Encoded</u>
599..1458	ampR
4123..3998	attR1
4372..5031	CmR
5151..5235	inactivated ccdA
5373..5678	ccdB
5719..5843	attR2

```

1  TTCACTGGCC  GTCGTTTTAC  AACGTCGTGA  CTGGGAAAAC  CCTGGCGTTA  CCCAACTTAA
61  TCGCCTTGCA  GCACATCCCC  CTTTCGCCAG  CTGGCGTAAT  AGCGAAGAGG  CCCGCACCGA
121  TCGCCCTTCC  CAACAGTTGC  GCAGCCTGAA  TGGCGAATGG  CGCCTGATGC  GGTATTTTCT
181  CCTTACGCAT  CTGTGCGGTA  TTTACACCG  CATATGGTGC  ACTCTCAGTA  CAATCTGCTC
241  TGATGCCGCA  TAGTTAAGCC  AGCCCCGACA  CCCGCCAACA  CCCGCTGACG  CGCCCTGACG
301  GGCTTGTCTG  CTCCCGGCAT  CCGCTTACAG  ACAAGCTGTG  ACCGTCTCCG  GGAGCTGCAT
361  GTGTCAGAGG  TTTTCACCGT  CATCACCGAA  ACGCGCGAGA  CGAAAGGGCC  TCGTGATACG
421  CCTATTTTTA  TAGGTAAATG  TCATGATAAT  AATGGTTTCT  TAGACGTCAG  GTGGCACTTT
481  TCGGGGAAAT  GTGCGCGGAA  CCCCTATTTG  TTTATTTTTC  TAAATACATT  CAAATATGTA
541  TCCGCTCATG  AGACAATAAC  CCTGATAAAT  GCTTCAATAA  TATTGAAAAA  GGAAGAGTAT
601  GAGTATTCAA  CATTTCCGTG  TCGCCCTTAT  TCCCTTTTTT  GCGGCATTTT  GCCTTCCTGT
661  TTTTGCTCAC  CCAGAAACGC  TGGTGAAAGT  AAAAGATGCT  GAAGATCAGT  TGGGTGCACG
721  AGTGGGTTAC  ATCGAACTGG  ATCTCAACAG  CGGTAAGATC  CTTGAGAGTT  TTCGCCCGA
781  AGAACGTTTT  CCAATGATGA  GCACTTTTAA  AGTTCTGCTA  TGTGGCGCGG  TATTATCCCG
841  TATTGACGCC  GGGCAAGAGC  AACTCGGTCT  CCGCATACAC  TATTCTCAGA  ATGACTTGGT
901  TGAGTACTCA  CCAGTCACAG  AAAAGCATCT  TACGGATGGC  ATGACAGTAA  GAGAATTATG
961  CAGTGCTGCC  ATAACCATGA  GTGATAACAC  TCGCGCCAAC  TTACTTCTGA  CAACGATCGG
1021  AGGACCGAAG  GAGCTAACCG  CTTTTTTTGA  CAACATGGGG  GATCATGTAA  CTCGCCTTGA
1081  TCGTTGGGAA  CCGGAGCTGA  ATGAAGCCAT  ACCAAACGAC  GAGCGTGACA  CCACGATGCC
1141  TGTAGCAATG  GCAACAACGT  TGCGCAAACT  ATTAAGTGGC  GAACTACTTA  CTCTAGCTTC
1201  CCGGCAACAA  TTAATAGACT  GGATGGAGGC  GGATAAAGTT  GCAGGACCAC  TTCTGCGCTC
1261  GGCCCTTCCG  GCTGGCTGGT  TTATTGCTGA  TAAATCTGGA  GCCGGTGAGC  GTGGGTCTCG
1321  CGGTATCATT  GCAGCACTGG  GGCCAGATGG  TAAGCCCTCC  CGTATCGTAG  TTATCTACAC
1381  GACGGGGAGT  CAGGCAACTA  TGGATGAACG  AAATAGACAG  ATCGCTGAGA  TAGGTGCCTC
1441  ACTGATTAAG  CATTGGTAAC  TGTCAGACCA  AGTTTACTCA  TATATACTTT  AGATTGATTT
1501  AAAACTTCAT  TTTTAATTTA  AAAGGATCTA  GGTGAAGATC  CTTTTTGATA  ATCTCATGAC
1561  CAAAATCCCT  TAACGTGAGT  TTTTCGTTCC  CTGAGCGTCA  GACCCCGTAG  AAAAGATCAA
1621  AGGATCTTCT  TGAGATCCTT  TTTTCTGCG  CGTAATCTGC  TGCTTGCAAA  CAAAAAACC
1681  ACCGCTACCA  GCGGTGGTTT  GTTGCCGGA  TCAAGAGCTA  CCAACTCTTT  TTCCGAAGGT
1741  AACTGGCTTC  AGCAGAGCGC  AGATACCAAA  TACTGTTCTT  CTAGTGTAGC  CGTAGTTAGG
1801  CCACCACTTC  AAGAACTCTG  TAGACCGGCC  TACATACCTC  GCTCTGCTAA  TCCTGTTACC
1861  AGTGGCTGCT  GCCAGTGGCG  ATAAGTCGTG  TCTTACCGGG  TTGGACTCAA  GACGATAGTT
1921  ACCGGATAAG  GCGCAGCGGT  CGGGCTGAAC  GGGGGGTTTC  TGCACACAGC  CCAGCTTGGA
1981  GCGAACGACC  TACACCGAAC  TGAGATACCT  ACAGCGTGAG  CATTGAGAAA  GCGCCACGCT
2041  TCCCGAAGGG  AGAAAGGCGG  ACAGGTATCC  GGTAAGCGGC  AGGGTCGGAA  CAGGAGAGCG
2101  CACGAGGGAG  CTTCCAGGGG  GAAACGCCTG  GTATCTTTAT  AGTCCTGTCT  GGTTTCGCCA
2161  CCTCTGACTT  GAGCGTCGAT  TTTTGTGATG  CTCGTCAGGG  GGGCGGAGCC  TATGGAAAAA
2221  CGCCAGCAAC  GCGGCCTTTT  TACGGTTCCT  GGCTTTTTC  TGGCCTTTTG  CTCACATGTT
2281  CTTTCTGCG  TTATCCCCTG  ATTCTGTGGA  TAACCGTATT  ACCGCCTTGT  AGTGAGCTGA
2341  TACCGCTCGC  CGCAGCCGAA  CGACCGAGCG  CAGCGAGTCA  GTGAGCGAGG  AAGCGGAAGA
2401  GCGCCCAATA  CGCAAACCGC  CTCTCCCGC  GCGTTGGCCG  ATTCATTAAT  GCAGCTGGCA
2461  CGACAGGTTT  CCCGACTGGA  AAGCGGGCAG  TGAGCGCAAC  GCAATTAATG  TGAGTTAGCT
2521  CACTCATTAG  GCACCCAGG  CTTTACACTT  TATGCTTCCG  GCTCGTATGT  TGTGTGGAAT
2581  TGTGAGCGGA  TAACAATTTT  ACACAGGAAA  CAGCTATGAC  CATGATTACG  CCAAGCTTGG
2641  CTGCAGGTGA  TGATTATCAG  CCAGCAGAGA  TTAAGGAAAA  CAGACAGGTT  TATTGAGCGC
2701  TTATCTTTCC  CTTTATTTTT  GCTGCGGTAA  GTCGCATAAA  AACCATTCTT  CATAATTCAA-

```

FIGURE 33B

82/240

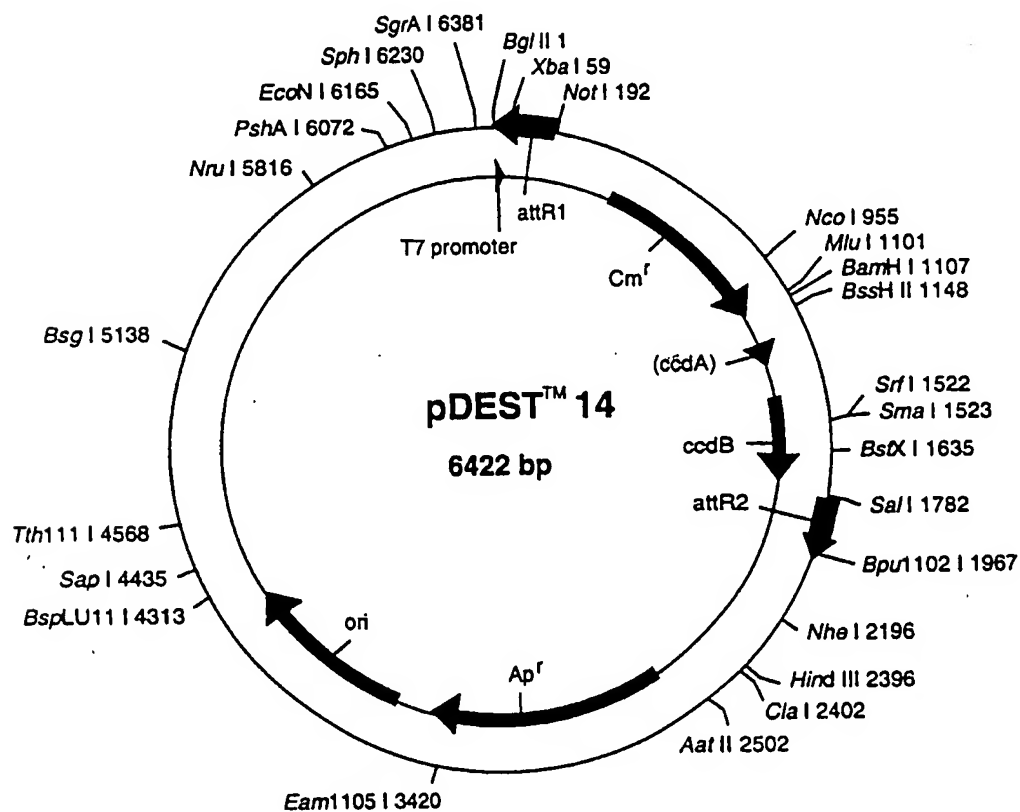
2761 TCCATTTACT ATGTTATGTT CTGAGGGGAG TGAAAATTCC CCTAATTCTGA TGAAGATTCT  
2821 TGCTCAATTG TTATCAGCTA TGCGCCGACC AGAACACCTT GCCGATCAGC CAAACGTCTC  
2881 TTCAGGCCAC TGA CTAGCGA TAACTTTCCC CACAACGGAA CAACTCTCAT TGCATGGGAT  
2941 CATTGGGTAC TGTGGGTTTA GTGGTTGTAA AAACACCTGA CCGCTATCCC TGATCAGTTT  
3001 CTTGAAGGTA AACTCATCAC CCCCAAGTCT GGCTATGCAG AAATCACCTG GCTCAACAGC  
3061 CTGCTCAGGG TCAACGAGAA TTAACATTCC GTCAGGAAAG CTTGGCTTGG AGCCTGTTGG  
3121 TGCGGTCATG GAATTACCTT CAACCTCAAG CCAGAATGCA GAATCACTGG CTTTTTGTGG  
3181 TGTGCTTACC CATCTCTCCG CATCACCTTT GGTAAAGGTT CTAAGCTTAG GTGAGAACAT  
3241 CCCTGCCTGA ACATGAGAAA AAACAGGGTA CTCATACTCA CTTCTAAGTG ACGGCTGCAT  
3301 ACTAACCGCT TCATACATCT CGTAGATTTT TCTGGCGATT GAAGGGCTAA ATTCTTCAAC  
3361 GCTAACTTTG AGAATTTTTG CAAGCAATGC GCGGTTATAA GCATTTAATG CATTGATGCC  
3421 ATTAAATAAA GCACCAACGC CTGACTGCCC CATCCCCATC TTGTCTGCGA CAGATTCTCTG  
3481 GGATAAGCCA AGTTCATTTT TCTTTTTC ATAAATTGCT TTAAGGCGAC GTGCGTCTCTC  
3541 AAGCTGCTCT TGTGTTAATG GTTTCTTTT TGTGCTCATA CGTTAAATCT ATCACCGCCAA  
3601 GGGATAAATA TCTAACACCG TGCGTGTGTA CTATTTTACC TCTGGCGGTG ATAATGGTTG  
3661 CATGTACTAA GGAGGTTGTA TGGAACAACG CATAACCTG AAAGATTATG CAATGCGCTT  
3721 TGGGCAAAACC AAGACAGCTA AAGATCTCTC ACCTACCAA CAATGCCCC CTGCAAAAAA  
3781 TAAATTCATA TAAAAACAT ACAGATAACC ATCTGCGGTG ATAAATTATC TCTGGCGGTG  
3841 TTGACATAAA TACCACTGGC GGTGATACTG AGCACATCAG CAGGACGCAC TGACCACCAT  
3901 GAAGGTGACG CTCTTAAAAA TTAAGCCCTG AAGAAGGGCA GCATTCAAAG CAGAAGGCTT  
3961 TGGGGTGTGT GATACGAAAC GAAGCATTGG GATCATCACA AGTTTGTACA AAAAAGCTGA  
4021 ACGAGAAACG TAAATGATA TAAATATCAA TATATTAAAT TAGATTTTGC ATAAAAAACA  
4081 GACTACATAA TACTGTAAAA CACAACATAT CCAGTCACTA TGGCGGCCGC TAAGTTGGCA  
4141 GCATCACCCG ACGCACTTTG CGCCGAATAA ATACCTGTGA CGGAAGATCA CTTCCGAGAA  
4201 TAAATAAATC CTGGTGTCCC TGTGATACC GGAAGCCCT GGGCCAATT TTGGCGAAAA  
4261 TGAGACGTTG ATCGGCACGT AAGAGTTCC AACTTTCACC ATAATGAAAT AAGATCACTA  
4321 CCGGGCGTAT TTTTGTAGTT ATCGAGATT TCAGGAGCTA AGGAAGCTAA AATGGAGAAA  
4381 AAAATCACTG GATATACCAC CGTTGATATA TCCCAATGGC ATCGTAAAGA ACATTTTGAG  
4441 GCATTTTCAGT CAGTTGCTCA ATGTACCTAT AACCAGACCG TTCAGCTGGA TATTACGCC  
4501 TTTTAAAGA CCGTAAAGAA AAATAAGCAC AAGTTTATC CGGCCTTTAT TCACATTCTT  
4561 GCCCGCTGA TGAATGCTCA TCCGGAATTC CGTATGGCAA TGAAAGACGG TGAGCTGGTG  
4621 ATATGGGATA GTGTTACCCC TTGTTACACC GTTTTCCATG AGCAAAGTGA AACGTTTCA  
4681 TCGCTCTGGA GTGAATACCA CGACGATTTC CGGCAGTTTC TACACATATA TTCGCAAGAT  
4741 GTGGCGTGT ACCTGTAAAA CCTGGCCTAT TTCCCTAAAG GGTATTATGA GAATATGTTT  
4801 TTCGTCTCAG CCAATCCCTG GGTGAGTTTC ACCAGTTTGT ATTTAAACGT GGCCAATATG  
4861 GACAACTTCT TCGCCCCCGT TTTACCATG GGCAAATATT ATACGCAAGG CGACAAGGTG  
4921 CTGATGCCGC TGGCGATTCA GGTTCATCAT GCCGTCTGTG ATGGCTTCCA TGTCCGCGA  
4981 ATGCTTAATG AATTACAACA GTACTGCGAT GAGTGGCAGG GCGGGGCGTA AACGCGTGA  
5041 TCCGGCTTAC TAAAGCCAG ATAACAGTAT GCGTATTGTC GCGCTGATTT TTGCGGTATA  
5101 AGAATATATA CTGATATGTA TACCCGAAGT ATGTCAAAAA GAGGTGTGCT ATGAAGCAGC  
5161 GTATTACAGT GACAGTTGAC AGCGACAGCT ATCAGTTGCT CAAGGCATAT ATGATGTCAA  
5221 TATCTCCGGT CTGGTAAGCA CAACCATGCA GAATGAAGCC CGTCGTCTGC GTGCCGAACG  
5281 CTGGAAAGCG GAAATCAGG AAGGGATGGC TGAGGTCGCC CGGTTTATTG AAATGAACGG  
5341 CTCTTTTGCT GACGAGAACA GGGACTGGTG AAATGCAGTT TAAGGTTTAC ACCTATAAAA  
5401 GAGAGAGCCG TTATCGTCTG TTTGTGGATG TACAGAGTGA TATTATTGAC ACGCCCGGGC  
5461 GACGGATGGT GATCCCCCTG GCCAGTGCAC GTCTGCTGTC AGATAAAGTC TCCCGTGAAC  
5521 TTTACCCGGT GGTGCATATC GGGGATGAAA GCTGGCGCAT GATGACCACC GATATGGCCA  
5581 GTGTGCCGGT CTCCGTATC GGGGAAGAAG TGGCTGATCT CAGCCACCGC GAAATGACA  
5641 TCAAAAACGC CATTAACTG ATGTTCTGGG GAATATAAAT GTCAGGCTCC GTTATACACA  
5701 GCCAGTCTGC AGGTCGACCA TAGTGACTGG ATATGTTGTG TTTTACAGTA TTATGTAGTC  
5761 TGTTTTTTAT GCAAAATCTA ATTTAATATA TTGATATTTA TATCATTTTA CGTTTCTCGT  
5821 TCAGCTTTCT TGTACAAAGT GGTGATAA

FIGURE 33C



Figure 34A: pDEST14 Native Protein Expression in *E. coli*, T7 Promoter

3961 tgccggccac gatgcgtccg gcgtagagga tcgagatctc gatcccgcca aattaatagc  
 acggccgggtg ctaccgaggc cgcattctct agctctagag ctaggggcgct ttaattatgc  
 4021 // actcactata gggagaccac aacggtttcc ctctagatca caagtttcta caaaaaagct  
 // tgagtgatat ccctctgggtg ttgccaaagg gagatctagt gttcaaacat gttttttcga



84/240

## pDEST14 6422 bp (rotated to position 4000)

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>	
185..61		attR1	
435..1094		CmR	
1214..1298		inactivated ccdA	
1436..1741		ccdB	
1782..1906		attR2	
2632..3489		ampR	
1	CGATCCCGCG AAATTAATAC GACTCACTAT AGGGAGACCA CAACGGTTTC CCTCTAGATC		
61	ACAAGTTTGT AAAAAAAGC TGAACGAGAA ACGTAAATG ATATAAATAT CAATATATTA		
121	AATTAGATTT TGCATAAAAA ACAGACTACA TAATACTGTA AAACACAACA TATCCAGTCA		
181	CTATGGCGGC CGCTAAGTTG GCAGCATCAC CCGACGCACT TTGCGCCGAA TAAATACCTG		
241	TGACGGAAGA TCACTTCGCA GAATAAATAA ATCCTGGTGT CCCTGTGTAT ACCGGGAAGC		
301	CCTGGGCCAA CTTTGGCGA AAATGAGACG TTGATCGGCA CGTAAGAGGT TCCAACTTTC		
361	ACCATAATGA AATAAGATCA CTACCGGGCG TATTTTTTGA GTTATCGAGA TTTTCAGGAG		
421	CTAAGGAAGC TAAAATGGAG AAAAAATCA CTGGATATAC CACCGTTGAT ATATCCCAAT		
481	GGCATCGTAA AGAACATTTT GAGGCATTTT AGTCAGTTGC TCAATGTACC TATAACCAGA		
541	CCGTTTCAGT GGATATTACG GCCTTTTAA AGACCGTAAA GAAAAATAAG CACAAGTTT		
601	ATCCGGCCTT TATTCACATT CTTGCCGCC TGATGAATGC TCATCCGGA TTCCGTATGG		
661	CAATGAAAGA CGGTGAGCTG GTGATATGGG ATAGTGTTC CCCTGTGTAT ACCGTTTTTC		
721	ATGAGCAAAC TGAAACGTTT TCATCGCTCT GGAGTGAATA CCACGACGAT TTCCGGCAGT		
781	TTCTACACAT ATATTCGCAA GATGTGGCGT GTTACGGTGA AAACCTGGCC TATTTCCCTA		
841	AAGGGTTTAT TGAGAATATG TTTTTCGTCT CAGCCAATCC CTGGGTGAGT TTCACCAGTT		
901	TTGATTTAAA CGTGGCCAAT ATGGACAAC TCTTCGCCCC CGTTTTCACC ATGGGCAAAT		
961	ATTATACGCA AGGCGACAAG GTGCTGATGC CGCTGGCGAT TCAGGTTTCAT CATGCCGTCT		
1021	GTGATGGCTT CCATGTCGGC AGAATGCTTA ATGAATTACA ACAGTACTGC GATGAGTGGC		
1081	AGGGCGGGGC GTAAACGCGT GGATCCGGCT TACTAAAAGC CAGATAACAG TATGCGTATT		
1141	TGCGCGCTGA TTTTTCGGT ATAAGAATAT ATACTGATAT GTATACCCGA AGTATGTCAA		
1201	AAAGAGGTGT GCTATGAAGC AGCGTATTAC AGTGACAGTT GACAGCGACA GCTATCAGTT		
1261	GCTCAAGGCA TATATGATGT CAATATCTCC GGTCTGGTAA GCACAACCAT GCAGAATGAA		
1321	GCCCGTCGTC TGCGTGCCGA ACGCTGGAAA GCGGAAAATC AGGAAGGGAT GGCTGAGGTC		
1381	GCCCGGTTTA TTGAAATGAA CGGCTCTTTT GCTGACGAGA ACAGGGACTG GTGAAATGCA		
1441	GTTTAAGGTT TACACCTATA AAAGAGAGAG CCGTTATCGT CTGTTTGTGG ATGTACAGAG		
1501	TGATATTATT GACACGCCCG GGCGACGGAT GGTGATCCCC CTGGCCAGTG CACGCTGTCT		
1561	GTCAGATAAA GTCTCCCGTG AACTTTACCC GGTGGTGCAT ATCGGGGATG AAAGCTGGCG		
1621	CATGATGACC ACCGATATGG CCAGTGTGCC GGTCTCCGTT ATCGGGGAAG AAGTGGCTGA		
1681	TCTCAGCCAC CGCGAAAATG ACATCAAAAA CGCCATTAAC CTGATGTTCT GGGGAATATA		
1741	AATGTCAAGC TCCCTTATAC ACAGCCAGTC TGCAGGTGCA CCATAGTGAC TGGATATGTT		
1801	GTGTTTTACA GTATTATGTA GTCTGTTTTT TATGCAAAAT CTAATTTAAT ATATTGATAT		
1861	TTATATCATT TTACGTTTCT CGTTCAGCTT TCTTGTAACA AGTGGTGATG ATCCGGCTGC		
1921	TAACAAAGCC CGAAAGGAAG CTGAGTTGGC TGCTGCCACC GCTGAGCAAT AACTAGCATA		
1981	ACCCCTTGGG GCCTCTAAAC GGGTCTTGAG GGGTTTTTTG CTGAAAGGAG GAACTATATC		
2041	CGGATATCCA CAGGACGGGT GTGGTCGCCA TGATCGCGTA GTCGATAGTG GCTCCAAGTA		
2101	GCGAAGCGAG CAGGACTGGG CGGCGGCCAA AGCGGTCGGA CAGTGCTCCG AGAACGGGTG		
2161	CGCATAGAAA TTGCATCAAC GCATATAGCG CTAGCAGCAC GCCATAGTGA CTGGCGATGC		
2221	TGTCGGAATG GACGATATCC CGCAAGAGGC CCGGCAGTAC CGGCATAACC AAGCCTATGC		
2281	CTACAGCATC CAGGGTGACG GTGCCGAGGA TGACGATGAG CGCATTGTGA GATTTTCATC		
2341	ACGGTGCCCTG ACTGCGTTAG CAATTTAACT GTGATAAACT ACCGCATTAA AGCTTATCGA		
2401	TGATAAGCTG TCAAACATGA GAATTTCTGA AGACGAAAGG GCCTCGTGAT ACGCTATT		
2461	TTATAGGTTA ATGTCATGAT AATAATGGTT TCTTAGACGT CAGGTGGCAC TTTTCGGGGA		
2521	AATGTGCGCG GAACCCCTAT TTGTTTATTT TTCTAAATAC ATTCAAATAT GTATCCGCTC		
2581	ATGAGACAAT AACCCCTGATA AATGCTTCAA TAATATTGAA AAAGGAAGAG TATGAGTATT		
2641	CAACATTTCC GTGTCGCCCT TATTCCCTTT TTTGCGGCAT TTTGCCTTCC TGTTTTTGCT		
2701	CACCCAGAAA CGCTGGTGAA AGTAAAAGAT GCTGAAGATC AGTTGGGTGC ACCAGTGGGT-		

Figure 34B

2761 TACATCGAAC TGGATCTCAA CAGCGGTAAG ATCCTTGAGA GTTTTCGCCC CGAAGAACGT  
2821 TTTC CAATGA TGAGCACTTT TAAAGTTCTG CTATGTGGCG CGGTATTATC CCGTGTTGAC  
2881 GCCGGGCAAG AGCAACTCGG TCGCCGCATA CACTATTCTC AGAATGACTT GGTGAGTAC  
2941 TCACCAGTCA CAGAAAAGCA TCTTACGGAT GGCATGACAG TAAGAGAATT ATGCAGTGCT  
3001 GCCATAACCA TGAGTGATAA CACTGCGGCC AACTTACTTC TGACAACGAT CGGAGGACCG  
3061 AAGGAGCTAA CCGCTTTTTT GCACAACATG GGGGATCATG TAACTCGCCT TGATCGTTGG  
3121 GAACCGGAGC TGAATGAAGC CATAACAAAC GACGAGCGTG ACACCACGAT GCCTGCAGCA  
3181 ATGGCAACAA CGTTGCGCAA ACTATTAACT GGCGAACTAC TTA CTCTAGC TTCCCGGCAA  
3241 CAATTAATAG ACTGGATGGA GGCGGATAAA GTTGCAGGAC CACTTCTGCG CTCGGCCCTT  
3301 CCGGCTGGCT GGT TTATTGC TGATAAATCT GGAGCCGGTG AGCGTGGGTC TCGCGGTATC  
3361 ATTGCAGCAC TGGGGCCAGA TGGTAAGCCC TCCCGTATCG TAGTTATCTA CACGACGGGG  
3421 AGTCAGGCAA CTATGGATGA ACGAAATAGA CAGATCGCTG AGATAGGTGC CTCACTGATT  
3481 AAGCATTGGT AACTGTCAGA CCAAGTTTAC TCATATATAC TTTAGATTGA TTTAAACTT  
3541 CATTTTAAAT TTA AAAGGAT CTAGGTGAAG ATCCTTTTTG ATAATCTCAT GACCAAAATC  
3601 CCTTAACGTG AGTTTTCGTT CCAC TGAGCG TCAGACCCCG TAGAAAAGAT CAAAGGATCT  
3661 TCTTGAGATC CTTTTTTTCT GCGCGTAATC TGCTGCTTGC AAACAAAAAA ACCACCGCTA  
3721 CCAGCGGTGG TTTGTTTGCC GGATCAAGAG CTACCAACTC TTTTCCGAA GGTAACTGGC  
3781 TTCAGCAGAG CGCAGATACC AAATACTGTC CTTCTAGTGT AGCCGTAGTT AGGCCACCAC  
3841 TTCAAGAACT CTGTAGCACC GCCTACATAC CTCGCTCTGC TAATCCTGTT ACCAGTGGCT  
3901 GCTGCCAGTG GCGATAAGTC GTGTCTTACC GGGTTGGACT CAAGACGATA GTTACCGGAT  
3961 AAGGCGCAGC GGTGCGGGCTG AACGGGGGGT TCGTGACAC AGCCAGCTT GGAGCGAAGC  
4021 ACCTACACCG AACTGAGATA CCTACAGCGT GAGCTATGAG AAAGCGCCAC GCTTCCCGAA  
4081 GGGAGAAAGG CGGACAGGTA TCCGGTAAGC GGCAAGGTCG GAACAGGAGA GCGCACGAGG  
4141 GAGCTTCCAG GGGGAAACGC CTGGTATCTT TATAGTCTG TCGGGTTTCG CCACCTCTGA  
4201 CTTGAGCGTC GATTTTGTG ATGCTCGTCA GGGGGGCGGA GCCTATGGAA AAACGCCAGC  
4261 AACGCGCCT TTTTACGGTT CCTGGCCTTT TGCTGGCCTT TTGCTCAGAT GTTCTTTCTT  
4321 GCGTTATCCC CTGATTCTGT GGATAACCGT ATTACCGCCT TTGAGTGAGC TGATACCGCT  
4381 CGCCGCAGCC GAACGACCGA GCGCAGCGAG TCAGTGAGCG AGGAAGCGGA AGAGCGCCTG  
4441 ATGCGGTATT TTCTCCTTAC GCATCTGTGC GGTATTTTAC ACCGCATATA TGGTGCACTC  
4501 TCCAGTACAAT CTGCTCTGAT GCCGCATAGT TAAGCCAGTA TACACTCCGC TATCGCTACG  
4561 TGACTGGGTC ATGGCTGCGC CCCGACACCC GCCAACACCC GCTGACGCGC CCTGACGGGC  
4621 TTGTCTGCTC CCGGCATCCG CTTACAGACA AGCTGTGACC GTCTCCGGA GCTGCATGTG  
4681 TCAGAGGTTT TCACCGTCAT CACCGAAACG CGCGAGGCAG CTGCGGTAAA GCTCATCAGC  
4741 GTGGTCTGTA AGCGATTAC AGATGTCTGC CTGTTTATCC GCGTCCAGCT CGTTGAGTTT  
4801 CTCCAGAAGC GTTAATGTCT GGCTTCTGAT AAAGCGGGCC ATGTTAAGGG CGGTTTTTTC  
4861 CTGTTTGGTC ACTGATGCCT CCGTGTAAGG GGGATTTCTG TTCATGGGG TAATGATACC  
4921 GATGAAACGA GAGAGGATGC TCACGATACG GGTACTGAT GATGAACATG CCCGGTTACT  
4981 GGAACGTTGT GAGGGTAAAC AACTGGCGGT ATGGATGCGG CGGGACCAGA GAAAAATCAC  
5041 TCAGGGTCAA TGCCAGCGCT TCGTTAATAC AGATGTAGGT GTTCCACAGG GTAGCCAGCA  
5101 GCATCCTGCG ATGCAGATCC GGAACATAAT GGTGCAGGGC GCTGACTTCC GCGTTTCCAG  
5161 ACTTTACGAA ACACGGAAAC CGAAGACCAT TCATGTTGTT GCTCAGGTCG CAGACGTTTT  
5221 GCAGCAGCAG TCGCTTCACG TTCGCTCGCG TATCGGTGAT TCATTCTGCT AACCAGTAAAG  
5281 GCAACCCCGC CAGCCTAGCC GGGTCCTCAA CGACAGGAGC ACGATCATGC GCACCCGTGG  
5341 CCAGGACCCA ACGCTGCCCG AGATGCGCCG CGTGCGGCTG CTGGAGATGG CGGACGCGAT  
5401 GGATATGTTT TGCCAAGGGT TGGTTTGCGC ATTCACAGTT CTCCGCAAGA ATTGATTGGC  
5461 TCCAATTCTT GGAGTGGTGA ATCCGTTAGC GAGGTGCCGC CGGCTTCCAT TCAGGTCGAG  
5521 GTGGCCCGGC TCCATGCACC GCGACGCAAC GCGGGGAGGC AGACAAGGTA TAGGGCGCGC  
5581 CCTACAATCC ATGCCAACCC GTTCCATGTG CTCGCCGAGG CGGCATAAAT CGCCGTGACG  
5641 ATCAGCGGTC CAGTGATCGA AGTTAGGCTG GTAAGAGCCG CGAGCGATCC TTGAAGCTGT  
5701 CCCTGATGGT CGTCATCTAC CTGCTGGAG AGCATGGCCT GCAACGCGGG CATCCCGATG  
5761 CCGCCGGAAG CGAGAAGAAT CATAATGGGG AAGGCCATCC AGCCTCGCGT CGCGAACGCC  
5821 AGCAAGACGT AGCCAGCGC GTCGGCCGCC ATGCCGCGCA TAATGGCCTG CTTCTCGCCG  
5881 AAACGTTTGG TGGCGGGACC AGTGACGAAG GCTTGAGCGA GGGCGTGCAA GATTCCGAAT  
5941 ACCGCAAGCG ACAGGCCGAT CATCGTCGCG CTCCAGCGAA AGCGGTCTCT GCCGAAAATG  
6001 ACCCAGAGCG CTGCCGGCAC CTGTCCTACG AGTTGCATGA TAAAGAAGAC AGTCATAAGT  
6061 GCGGCGACGA TAGTCATGCC CCGCGCCAC CGGAAGGAGC TGA CTGGGTT GAAGGCTCTC  
6121 AAGGGCATCG GTCGATCGAC GCTCTCCCTT ATGCGACTCC TGCAATTAGGA AGCAGCCAG  
6181 TAGTAGGTTG AGGCCGTTGA GCACCGCCCG CGCAAGGAAT GGTGCATGCA AGGAGATGGC-

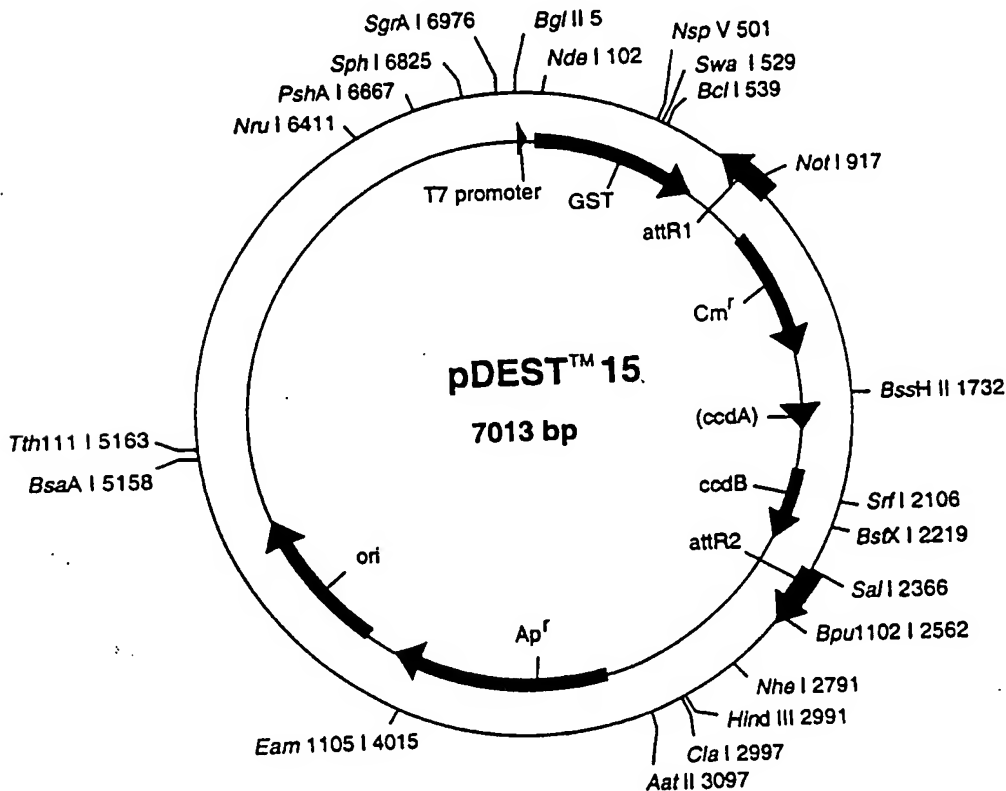
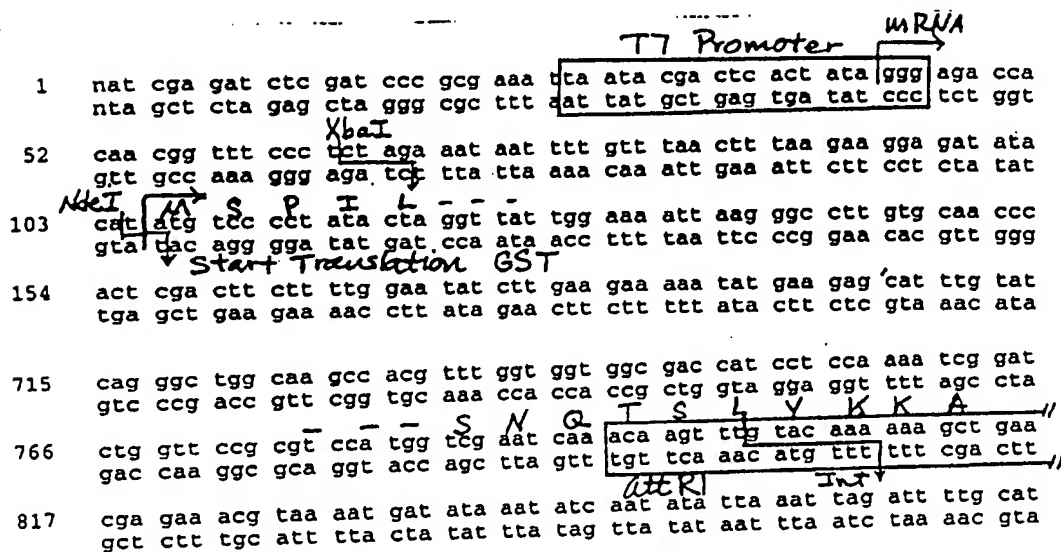
FIGURE 34C

86/240

6241 GCCCAACAGT CCCCCGGCCA CGGGGCCTGC CACCATACCC ACGCCGAAAC AAGCGTCAT  
6301 GAGCCCGAAG TGGCGAGCCC GATCTTCCCC ATCGGTGATG TCGGCGATAT AGGCGCCAGC  
6361 AACCGCACCT GTGGCGCCGG TGATGCCGGC CACGATGCGT CCGGCGTAGA GGATCGAGAT  
6421 CT

FIGURE 34D

**Figure 35A: pDEST15 Glutathione-S-transferase Fusion in *E. coli*, T7 Promoter**



88/240

## pDEST15 7013 bp

<u>Location (Base Nos.)</u>	<u>Gene Encoded</u>
108..776	GST
916..792	attR1
1025..1537	CmR
1804..1888	inactivated ccdA
2026..2331	ccdB
2372..2496	attR2
3233..4093	ampR

```

1  ATCGAGATCT CGATCCCGCG AAATTAATAC GACTCACTAT AGGGAGACCA CAACGGTTTC
61 CCTCTAGAAA TAATTTTGTG TAACTTTAAG AAGGAGATAT ACATATGTCC CCTATACTAG
121 GTTATTGGAA AATTAAGGCG CTTGTGCAAC CCACTCGACT TCTTTTGGAA TATCTTGAAG
181 AAAAATATGA AGAGCATTTG TATGAGCGCG ATGAAGGTGA TAAATGGCGA AACAAAAAGT
241 TTGAATTGGG TTGGAGTTT CCCAATCTTC CTTATTATAT TGATGGTGAT GTTAAATTAA
301 CACAGTCTAT GGCCATCATA CGTTATATAG CTGACAAGCA CAACATGTTG GGTGGTTGTC
361 CAAAAGAGCG TGCAGAGATT TCAATGCTTG AAGGAGCGGT TTTGGATATT AGATACGGTG
421 TTTGAGAAAT TGCATATAGT AAAGACTTTG AAACCTCTCA AGTTGATTTT CTTAGCAAGC
481 TACCTGAAAT GCTGAAAATG TTGGAAGATC GTTTATGTCA TAAAACATAT TTAAATGGTG
541 ATCATGTAAC CCATCCTGAC TTCATGTTGT ATGACGCTCT TGATGTTGTT TTATACATGG
601 ACCCAATGTG CCTGGATGCG TTCCCAAAAT TAGTTTGTGT TAAAAACGT ATTGAAGCTA
661 TCCACAAAT TGATAAGTAC TTGAAATCCA GCAAGTATAT AGCATGGCCT TTGAGGGCT
721 GGCAAGCCAC GTTTGGTGGT GGCGACCATC CTCCAAAATC GGATCTGGTT CCGGTCCTAT
781 GGTGCAATCA AACAAAGTTG TACAAAAAAG CTGAACGAGA AACGTAAAT GATATAAATA
841 TCAATATATT AAATTAGATT TTGCATAAAA AACAGACTAC ATAATACTGT AAAACACAAC
901 ATATCCAGTC ACTATGGCGG CCGCATTAGG CACCCCAGGC TTTACACTTT ATGCTTCCGG
961 CTCGTATAAT GTGTGGATTT TGAGTTAGGA TCCGTCGAGA TTTTCAGGAG CTAAGGAAGC
1021 TAAATGGAG AAAAAATCA CTGGATATAC CACCGTTGAT ATATCCCAAT GGCATCGTAA
1081 AGAACATTTT GAGGCATTTC AGTCAGTTGC TCAATGTACC TATAACCAGA CCGTTCAGCT
1141 GGATATTACG GCCTTTTAA AGACCGTAAA GAAAAATAAG CACAAGTTT ATCCGGCCTT
1201 TATTCACATT CTTGCCCGCC TGATGAATGC TCATCCGGAA TTCCGTATGG CAATGAAAGA
1261 CGGTGAGCTG GTGATATGGG ATAGTGTTCA CCCTTGTTAC ACCGTTTCC ATGAGCAAAC
1321 TGAAACGTTT TCATCGCTCT GGAGTGAATA CCACGACGAT TTCCGGCAGT TTCTACACAT
1381 ATATTCGCAA GATGTGGCGT GTTACGGTGA AAACCTGGCC TATTTCCCTA AAGGGTTTAT
1441 TGAGAATATG TTTTTCGTCT CAGCCAATCC CTGGGTGAGT TTCACCAAGT TTGATTTAA
1501 CGTGGCCAAT ATGGACAAC TCTTCGCCCC CGTTTTTACC ATGGGCAAAT ATTATACGCA
1561 AGGCGACAAG GTGCTGATGC CGCTGGCGAT TCAGGTTTCT CATGCCGCTT GTGATGGCTT
1621 CCATGTCCGC AGAATGCTTA ATGAATTACA ACAGTACTGC GATGAGTGGC AGGGCGGGGC
1681 GTAATCTAGA GGATCCGGCT TACTAAAAGC CAGATAACAG TATGCGTATT TGCGCGCTGA
1741 TTTTTCGGGT ATAAGAATAT ATACTGATAT GTATACCGA AGTATGTCAA AAAGAGGTGT
1801 GCTATGAAGC AGCGTATTAC AGTGACAGTT GACAGCGACA GCTATCAGTT GCTCAAGGCA
1861 TATATGATGT CAATATCTCC GGTCTGGTAA GCACAACCAT GCAGAATGAA GCCCGTCGTC
1921 TGCGTGCCGA ACGCTGGAAA GCGGAAATC AGGAAGGGAT GGCTGAGGTC GCCCGTTTAA
1981 TTGAAATGAA CGGCTCTTTT GCTGACGAGA ACAGGGACTG GTGAAATGCA GTTTAAGGTT
2041 TACACCTATA AAAGAGAGAG CCGTTATCGT CTGTTTGTGG ATGTACAGAG TGATATTATT
2101 GACACGCCCG GGCGACGGAT GGTGATCCCC CTGGCCAGTG CACGTCTGCT GTCAGATAAA
2161 GTCTCCCCGT AACTTTACCC GGTGGTGCAT ATCGGGGATG AAAGCTGGCG CATGATGACC
2221 ACCGATATGG CCAGTGTGCC GGTCTCCGTT ATCGGGGAAG AAGTGGCTGA TCTCAGCCAC
2281 CGCGAAAATG ACATCAAAAA CGCCATTAAC CTGATGTTCT GGGGAATATA AATGTCAGGC
2341 TCCCTTATAC ACAGCCAGTC TGCAGGTCGA CCATAGTGAC TGGATATGTT GTGTTTACA
2401 GTATTATGTA GTCTGTTTTT TATGCAAAAT CTAATTAAAT ATATTGATAT TTATATCATT
2461 TTACGTTTCT CGTTCAGCTT TCTTGTAACA AGTGGTTTGA TTCGACCCGG GATCCGGCTG
2521 CTAACAAAGC CCGAAAGGAA GCTGAGTTGG CTGCTGCCAC CGCTGAGCAA TAACTAGCAT
2581 AACCCCTTGG GGCCTCTAAA CGGGTCTTGA GGGGTTTTTT GCTGAAAGGA GGAACATAT
2641 CCGGATATCC ACAGGACGGG TGTGGTCGCC ATGATCGCGT AGTCGATAGT GGCTCAAAGT-

```

Figure 35B

89/240

2701 AGCGAAGCGA GCAGGACTGG GCGGCGGCCA AAGCGGTCGG ACAGTGCTCC GAGAACGGGT  
2761 GCGCATAGAA ATTGCATCAA CGCATATAGC GCTAGCAGCA CGCCATAGTG ACTGGCGATG  
2821 CTGTCCGAAT GGACGATATC CCGCAAGAGG CCCGGCAGTA CCGGCATAAC CAAGCCTATG  
2881 CCTACAGCAT CCAGGGTGAC GGTGCCGAGG ATGACGATGA GCGCATTGTT AGATTTCATA  
2941 CACGGTGCTT GACTGCGTTA GCAATTAAAC TGTGATAAAC TACCGCATT AAGCTTATCG  
3001 ATGATAAGCT GTCAAACATG AGAATTCTTG AAGACGAAAG GGCCTCGTGA TACGCCCTATT  
3061 TTTATAGGTT AATGTCATGA TAATAATGGT TTCTTAGACG TCAGGTGGCA CTTTTTCGGG  
3121 AAATGTGCGC GGAACCCCTA TTTGTTTATT TTTCTAAATA CATTCAAATA TGTATCCGCT  
3181 CATGAGACAA TAACCGTGAT AAATGCTTCA ATAATATTGA AAAAGGAAGA GTATGAGTAT  
3241 TCAACATTTT CGTGTGCGCC TTATTCCCTT TTTTGGCGCA TTTTGCCTTC CTGTTTTTGC  
3301 TCACCCAGAA ACGCTGGTGA AAGTAAAAGA TGCTGAAGAT CAGTTGGGTG CACGAGTGGG  
3361 TTACATCGAA CTGGATCTCA ACAGCGGTAA GATCCTTGAG AGTTTTCCGC CCGAAGAACG  
3421 TTTTCCAATG ATGAGCACTT TAAAGTTCT GCTATGTGGC GCGGTATTAT CCCGTGTTGA  
3481 CGCCGGGCAA GAGCAACTCG GTCGCCGCAT AACTATTCT CAGAATGACT TGGTTGAGTA  
3541 CTCACCACTC ACAGAAAAGC ATCTTACGGA TGGCATGACA GTAAGAGAA GTACAGTGC  
3601 TGCCATAACC ATGAGTGATA ACATGCGGC CAACTTACTT CTGACAACGA TCGGAGGACC  
3661 GAAGGAGCTA ACCGCTTTTT TGCACAACAT GGGGGATCAT GTAACTCGCC TTGATCGTTG  
3721 GGAACCGGAG CTGAATGAAG CCATACCAA CGACGAGCGT GACACCACGA TGCCTGCAGC  
3781 AATGGCAACA ACGTTGCGCA AACTATTAACT TGGCGAACTA CTTACTCTAG CTTCCCGCA  
3841 ACAATTAATA GACTGGATGG AGGCGGATAA AGTTGCAGGA CCACTTCTGC GCTCGGCCCT  
3901 TCCGGCTGGC TGGTTTATTG CTGATAAATC TGGAGCCGGT GAGCGTGGGT CTCGCGGTAT  
3961 CATTGCAGCA CTGGGGCCAG ATGGTAAGCC CTCCCGTATC GTAGTTATCT ACACGACGGG  
4021 GAGTCAGGCA ACTATGGATG AACGAAATAG ACAGATCGCT GAGATAGGT CCTCACTGAT  
4081 TAAGCATTGG TAACTGTCAG ACCAAGTTTA CTCATATATA CTTTAGATTG ATTTAAACT  
4141 TCATTTTTAA TTTAAAAGGA TCTAGGTGAA GATCCTTTTT GATAATCTCA TGACCAAAAT  
4201 CCCTTAACGT GAGTTTTCGT TCCACTGAGC GTCAGACCCC GTAGAAAAGA TCAAAGGATC  
4261 TTCTTGAGAT CCTTTTTTTC TGC CGCTAAT CTGCTGCTTG CAAACAAAAA AACCACCGCT  
4321 ACCAGCGGTG GTTTGTTTGC CGGATCAAGA GCTACCAACT CTTTTCCGA AGGTAACCTG  
4381 CTTACAGCAG GCGCAGATAC CAAATACTGT CTTCTAGTG TAGCCGTAGT TAGGCCACCA  
4441 TTCAAGAAC TCTGTAGCAC CGCCTACATA CCTCGCTCTG CTAATCCTGT TACCAGTGGC  
4501 TGCTGCCAGT GCGGATAAGT CGTGTCTTAC CGGGTTGGAC TCAAGACGAT AGTTACCGGA  
4561 TAAGGCGCAG CGGTGCGGCT GAACGGGGGG TTCGTGCACA CAGCCCAGCT TGGAGCGAAC  
4621 GACCTACACC GAACTGAGAT ACCTACAGCG TGAGCTATGA GAAAGCGCCA CGCTTCCCGA  
4681 AGGGAGAAAG GCGGACAGGT ATCCGGTAAG CCGCAGGGTC GGAACAGGAG AGCGCACGAG  
4741 GGAGCTTCCA GGGGGAACG CCTGGTATCT TTATAGTCCT GTCGGGTTTC GCCACCTCTG  
4801 ACTTGAGCGT CGATTTTTGT GATGCTCGTC AGGGGGGCGG AGCCTATGGA AAAAGCCAG  
4861 CAACGCGGCC TTTTACGGT TCCTGGCCTT TTGCTGGCCT TTGCTCACA TGTCTTTCC  
4921 TGCGTTATCC CCTGATTCTG TGGATAACCG TATTACCGCC TTTGAGTGAG CTGATACCGC  
4981 TCGCCGCAGC CGAACGACCG AGCGCAGCGA GTCAGTGAGC GAGGAAGCGG AAGAGCGCCT  
5041 GATGCGGTAT TTTCTCCTTA CGCATCTGTG CCGTATTTCA CACCGCATAT ATGGTGCAT  
5101 CTCAGTACAA TCTGCTCTGA TGCCGCATAG TTAAGCCAGT ATACACTCCG CTATCGCTAC  
5161 GTGACTGGGT CATGGCTGCG CCCCACACCG CGCCAACACC CGCTGACCGC CCCTGACGGG  
5221 CTTGTCTGCT CCCGGCATCC GCTTACAGAC AAGCTGTGAC CGTCTCCGGG AGCTGCATGT  
5281 GTCAGAGGTT TTCACCGTCA TCACCGAAAC GCGCGAGGCA GCTGCGGTAA AGCTCATCAG  
5341 CGTGGTCGTG AAGCGATTCA CAGATGTCTG CCTGTTTCATC CGCGTCCAGC TCGTTGAGTT  
5401 TCTCCAGAAG CGTTAATGTC TGGCTTCTGA TAAAGCGGGC CATGTTAAGG GCGGTTTTTT  
5461 CCTGTTTGGT CACTGATGCC TCCGTGTAAG GGGGATTCT GTTCATGGGG GTAATGATAC  
5521 CGATGAAACG AGAGAGGATG CTCACGATAC GGGTTACTGA TGATGAACAT GCCCGGTTAC  
5581 TGAACGTTG TGAGGGTAA CAACTGGCGG TATGGATGCG GCGGGACCAG AGAAAAATCA  
5641 CTCAGGGTCA ATGCCAGCGC TTCGTTAATA CAGATGTAGG TGTCCACAG CGTAGCCAGC  
5701 AGCATCCTGC GATGCGATC CGGAACATAA TGGTGAGGG CGCTGACTTC CGCGTTTTCA  
5761 GACTTTACGA AACACGAAA CCGAAGACCA TTCATGTTGT TGCTCAGGTC GCAGACGTTT  
5821 TGCAGCAGCA GTCGCTTCAC GTTCGCTCGC GTATCGGTGA TTCATTCTGC TAACCAGTAA  
5881 GGCAACCCCG CCAGCCTAGC CGGTCTCTCA ACGACAGGAG CACGATCATG CGCACCCGTG  
5941 GCCAGGACCC AACGCTGCCC GAGATGCGCC GCGTGCGGCT GCTGGAGATG GCGGACGCGA  
6001 TGGATATGTT CTGCCAAGGG TTGGTTTTCG CATTACAGT TCTCCGCAAG AATTGATTGG  
6061 CTCCAATTCT TGGAGTGGTG AATCCGTTAG CGAGGTGCCG CCGGCTTCCA TTCAGTCCA  
6121 GGTGGCCCGG CTCCATGCAC CGCGACGCAA CGCGGGGAGG CAGACAAGGT ATAGGGCGGC-

FIGURE 35C

90/240

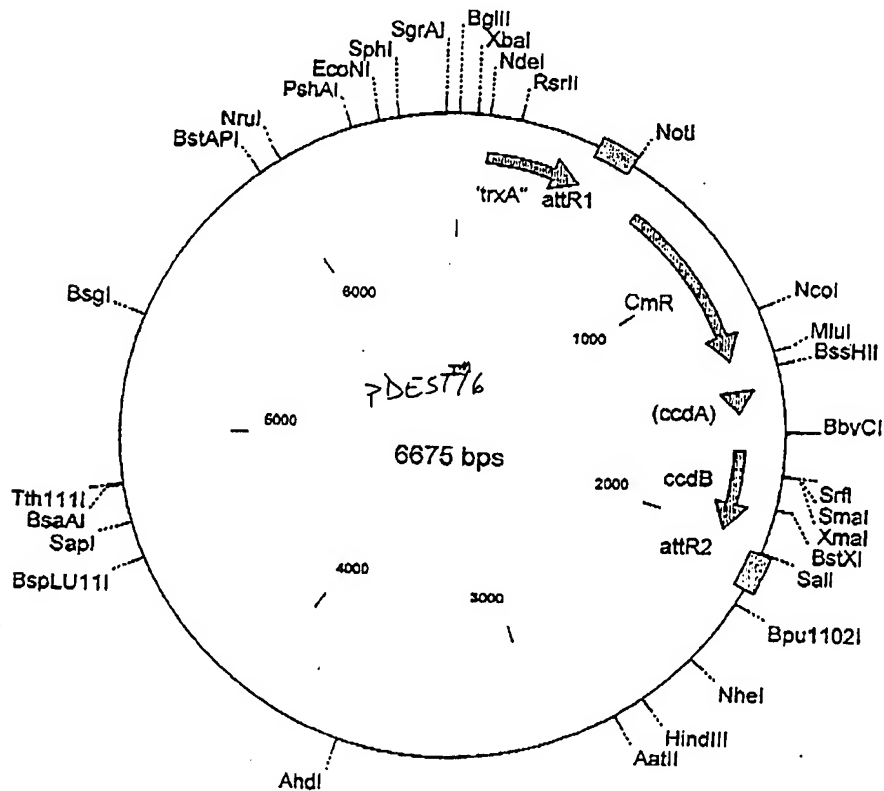
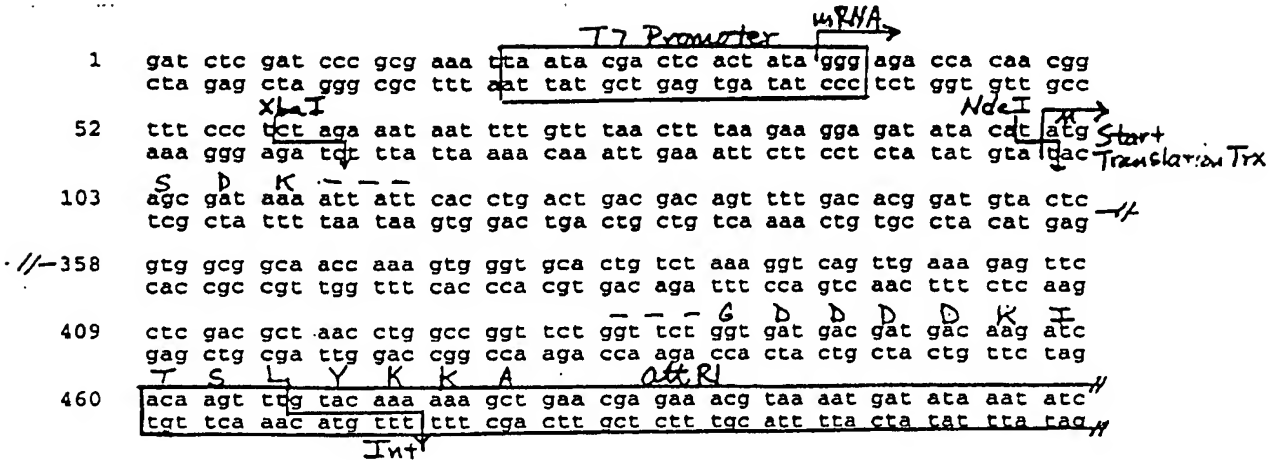
6181 GCCTACAATC CATGCCAACC CGTTCCATGT GCTCGCCGAG GCGGCATAAA TCGCCGTGAC  
6241 GATCAGCGGT CCAGTGATCG AAGTTAGGCT GGTAAGAGCC GCGAGCGATC CTTGAAGCTG  
6301 TCCCTGATGG TCGTCATCTA CCTGCCTGGA CAGCATGGCC TGCAACGCGG GCATCCCGAT  
6361 GCCGCCGGAA GCGAGAAGAA TCATAATGGG GAAGGCCATC CAGCCTCGCG TCGCGAACGC  
6421 CAGCAAGACG TAGCCCAGCG CGTCGGCCGC CATGCCGGCG ATAATGGCCT GCTTCTCGCC  
6481 GAAACGTTTG GTGGCGGGAC CAGTGACGAA GGCTTGAGCG AGGGCGTGCA AGATTCCGAA  
6541 TACCGCAAGC GACAGGCCGA TCATCGTCGC GCTCCAGCGA AAGCGGTCCT CGCCGAAAA  
6601 GACCCAGAGC GCTGCCGGCA CCTGTCCTAC GAGTTGCATG ATAAAGAAGA CAGTCATAAG  
6661 TGCGGCGACG ATAGTCATGC CCCGCGCCCA CCGGAAGGAG CTGACTGGGT TGAAAGGCTCT  
6721 CAAGGGCATC GGTGATCGA CGCTCTCCCT TATGCGACTC CTGCATTAGG AAGCAGCCCA  
6781 GTAGTAGGTT GAGGCCGTTG AGCACCGCCG CCGCAAGGAA TGGTGCATGC AAGGAGATGG  
6841 CGCCCAACAG TCCCCCGGCC ACGGGGCTG CCACCATACC CACGCCGAAA CAAGCGCTCA  
6901 TGAGCCCGAA GTGGCGAGCC CGATCTTCCC CATCGGTGAT GTCGGCGATA TAGGCGCCAG  
6961 CAACCGCACC TGTGGCGCCG GTGATGCCGG CCACGATGCG TCCGGCGTAG AGG

FIGURE 351)



9/1/240

Figure 36A: pDEST16

Thioredoxin N-Fusion Protein  
in E. coli with T7 Promoter

92/240

## pDEST16 6675 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
104..457		trxA
585..461		attR1
694..1353		CmR
1473..1557		inactivated ccdA
1695..2000		ccdB
2041..2165		attR2
1	AGATCTCGAT CCCGCGAAAT TAATACGACT CACTATAGGG AGACCACAAC GGTTTCCCTC	
61	TAGAAATAAT TTTGTTTAACT TTTAAGAAGG AGATATACAT ATGAGCGATA AAATTATTCA	
121	CCTGACTGAC GACAGTTTTG ACACGGATGT ACTCAAAGCG GACGGGGCGA TCCTCGTCGA	
181	TTTCTGGGCA GAGTGGTGCG GTCCGTGCAA AATGATCGCC CCGATTCTGG ATGAAATCGC	
241	TGACGAATAT CAGGGCAAAC TGACCGTTGC AAAACTGAAC ATCGATCAAA ACCCTGGCAC	
301	TGCGCCGAAA TATGGCATCC GTGGTATCCC GACTCTGCTG CTGTTCAAAA ACGGTGAAGT	
361	GGCGGCAACC AAAGTGGGTG CACTGTCTAA AGGTCAGTTG AAAGAGTTCC TCGACGCTAA	
421	CCTGGCCGGT TCTGGTTCTG GTGATGACGA TGACAAGATC ACAAGTTTGT ACAAAAAAGC	
481	TGAACGAGAA ACGTAAAATG ATATAAATAT CAATATATTA AATTAGATTT TGCATAAAAA	
541	ACAGACTACA TAATACTGTA AAACACAACA TATCCAGTCA CTATGGCGGC CGCATTAGGC	
601	ACCCAGGCT TTACACTTTA TGCTCCGGC TCGTATAATG TGTGGATTTT GAGTTAGGAT	
661	CCGGCGAGAT TTTCAGGAGC TAAGGAAGCT AAAATGGAGA AAAAAATCAC TGGATATACC	
721	ACCGTTGATA TATCCCAATG GCATCGTAAA GAACATTTTG AGGCATTTCA GTCAGTTGCT	
781	CAATGTACCT ATAACCAGAC CGTTCAGCTG GATATTACGG CCTTTTAA GACCGTAAAG	
841	AAAAATAAGC ACAAGTTTAA TCCGGCCTTT ATTACATTC TTGCCCGCT GATGAATGCT	
901	CATCCGGAAT TCCGTATGGC AATGAAAGAC GGTGAGCTGG TGATATGGGA TAGTGTTCAC	
961	CCTTGTTACA CCGTTTCCA TGAGCAAAC GAAACGTTT CATCGCTCTG GAGTGAATAC	
1021	CACGACGATT TCCGGCAGTT TCTACACATA TATTCGCAAG ATGTGGCGTG TTACGGTGAA	
1081	AACCTGGCCT ATTTCCCTAA AGGGTTTATT GAGAATATGT TTTTCGTCTC AGCCAATCCC	
1141	TGGGTGAGTT TCACCAAGTT TGATTTAAAC GTGGCCAATA TGGACAACCT CTTCGCCCCC	
1201	GTTTTTACCA TGGGCAAATA TTATACGCAA GCGACAAGG TGCTGATGCC GCTGGCGATT	
1261	CAGGTTTCATC ATGCCGCTCG TGATGGCTTC CATGTGCGCA GAATGCTTAA TGAATTACAA	
1321	CAGTACTGCG ATGAGTGGCA GGGCGGGGCG TAAACGCGTG GATCCGGCTT ACTAAAAGCC	
1381	AGATAACAGT ATGCGTATTT GCGCGCTGAT TTTTGCGGTA TAAGAATATA TACTGATATG	
1441	TATACCCGAA GTATGTCAAA AAGAGGTGTG CTATGAAGCA GCGTATTACA GTGACAGTTG	
1501	ACAGCGACAG CTATCAGTTG CTCAAGGCAT ATATGATGTC AATATCTCCG GTCTGGTAAG	
1561	ACAACCATG CAGAATGAAG CCCGTCGTCT GCGTGCCGAA CGCTGGAAAG CGGAAATCA	
1621	GGAAGGGATG GCTGAGGTCG CCGGTTTAT TGAATGAAC GGCTCTTTTG CTGACGAGAA	
1681	CAGGGACTGG TGAATGCAG TTTAAGGTTT ACACCTATAA AAGAGAGAGC CGTTATCGTC	
1741	TGTTTGTGGA TGTACAGAGT GATATTATTG ACACGCCCCG GCGACGGATG GTGATCCCCC	
1801	TGGCCAGTGC ACGTCTGCTG TCAGATAAAG TCTCCCGTGA ACTTTACCCG GTGGTGCATA	
1861	TCGGGGATGA AAGTGGCGC ATGATGACCA CCGATATGGC CAGTGTGCCG GTCTCCGTTA	
1921	TCGGGGAAGA AGTGGCTGAT CTCAGCCACC GCGAAAATGA CATCAAAAAC GCCATTAACC	
1981	TGATGTTCTG GGAATATAA ATGTCAGGCT CCCTTATACA CAGCCAGTCT GCAGGTCGAC	
2041	CATAGTGACT GGATATGTTG TGTTTTACAG TATTATGTAG TCTGTTTTTT ATGCAAAATC	
2101	TAATTTAATA TATTGATATT TATATCATTT TACGTTTCTC GTTCAGCTTT CTGTACAAA	
2161	GTGGTGATGA TCCGGCTGCT AACAAAGCCC GAAAGGAAGC TGAGTTGGCT GCTGCCACCG	
2221	CTGAGCAATA ACTAGCATAA CCCCTTGGGG CCTCTAAACG GGTCTTGAGG GGTTTTTTGC	
2281	TGAAAGGAGG AACTATATCC GGATATCCAC AGGACGGGTG TGGTCGCCAT GATCGCGTAG	
2341	TCGATAGTGG CTCCAAGTAG CGAAGCGAGC AGGACTGGGC GGCGGCCAAA GCGTCCGAC	
2401	AGTGCTCCGA GAACGGGTGC GCATAGAAAT TGCATCAACG CATATAGCGC TAGCAGCACG	
2461	CCATAGTGAC TGGCGATGCT GTCGGAATGG ACGATATCCC GCAAGAGGCC CGGCAGTACC	
2521	GGCATAACCA AGCCTATGCC TACAGCATCC AGGGTGACGG TGCCGAGGAT GACGATGAGC	
2581	GCATTGTTAG ATTTTCATACA CCGTGCCCTGA CTGCGTTAGC AATTTAACTG TGATAAACTA	
2641	CCGATTAAA GCTTATCGAT GATAAGCTGT CAAACATGAG AATTCTTGAA GACGAAAGGG	
2701	CCTCGTGATA CGCCTATTTT TATAGGTTAA TGTCATGATA ATAATGGTTT CTTAGACGTC	
2761	AGGTGGCACT TTTCCGGGAA ATGTGCGCGG AACCCTATT TGTTTATTTT TCTAAATACA-	

Figure 36B

93/240

2821 TTCAAATATG TATCCGCTCA TGAGACAATA ACCCTGATAA ATGCTTCAAT AATATTGAAA  
 2881 AAGGAAGAGT ATGAGTATTC AACATTTCCG TGTCGCCCTT ATTCCCTTTT TTGCGGCATT  
 2941 TTGCCTTCCT GTTTTTGCTC ACCCAGAAAC GCTGGTGAAA GTAAAAGATG CTGAAGATCA  
 3001 GTTGGGTGCA CGAGTGGGTT ACATCGAACT GGATCTCAAC AGCGGTAAGA TCCTTGAGAG  
 3061 TTTTCGCCCC GAAGAACGTT TTCCAATGAT GAGCACTTTT AAAGTTCTGC TATGTGGCGC  
 3121 GGTATTATCC CGTGTGACG CCGGGCAAGA GCAACTCGGT CGCCGCATAC ACTATTCTCA  
 3181 GAATGACTTG GTTGAGTACT CACCAGTCAC AGAAAAGCAT CTTACGGATG GCATGACAGT  
 3241 AAGAGAATTA TGCAGTGCTG CCATAACCAT GAGTGATAAC ACTGCGGCCA ACTTACTTCT  
 3301 GACAACGATC GGAGGACCGA AGGAGCTAAC CGCTTTTGTG CACAACATGG GGGATCATGT  
 3361 AACTCGCCTT GATCGTTGGG AACCGGAGCT GAATGAAGCC ATACCAAACG ACAGAGCGTGA  
 3421 CACCACGATG CCTGCAGCAA TGGCAACAAC GTTGCAGCAA CTATTAACCTG GCGAACTACT  
 3481 TACTCTAGCT TCCCAGCAAC AATTAATAGA CTGGATGGAG GCGGATAAAG TTGCAGGACC  
 3541 ACTTCTGCGC TCGGCCCTTC CGGCTGGCTG GTTTATTGCT GATAAATCTG GAGCCGGTGA  
 3601 GCGTGGGTCT CGCGGTATCA TTGCAGCACT GGGGCCAGAT GGTAAGCCCT CCCGTATCGT  
 3661 AGTTATCTAC ACGACGGGGA GTCAGGCAAC TATGGATGAA CGAAATAGAC AGATCGCTGA  
 3721 GATACGGTGC TCACTGATTA AGCATTGGTA ACTGTCAGAC CAAGTTTACT CATATCATGT  
 3781 TTAGATTGAT TTAATACTTC ATTTTAAATT TAAAAGGATC TAGGTGAAGA TCCTTTTGTG  
 3841 TAATCTCATG ACCAAAATCC CTTAACGTGA GTTTTCGTTC CACTGAGCGT CAGACCCCGT  
 3901 AGAAAAGATC AAAGGATCTT CTTGAGATCC TTTTTCCTG CGCGTAATCT GCTGCTTGCA  
 3961 AACAAAAAAA CCACCGCTAC CAGCGGTGGT TTGTTTGCCG GATCAAGAGC TACCAACTCT  
 4021 TTTTCCGAAG GTAACGGCT TCAGCAGAGC GCAGATACCA AATACTGTCC TTCTAGTGTA  
 4081 GCCGTAGTTA GGCCACCACT TCAAGAACTC TGTAGCACC GCTACATACC TCGCTCTGCT  
 4141 AATCCTGTGA CCAGTGGCTG CTGCCAGTGG CGATAAGTCG TGTCTTACCG GGTGGACTC  
 4201 AAGACGATAG TTACCGGATA AGGCGCAGCG GTCGGGCTGA ACGGGGGGTT CGTGACACA  
 4261 GCCCAGCTTG GAGCGAACGA CCTACACCGA ACTGAGATAC CTACAGCGTG AGCTATGAGA  
 4321 AAGCGCCACG CTTCCCGAAG GGAGAAAGGC GGACAGGTAT CCGGTAAGCG GCAGGGTCGG  
 4381 AACAGGAGAG CGCAGGAGGG AGCTTCCAGG GGGAAACGCC TGGTATCTTT ATAGTCCTGT  
 4441 CGGGTTTCGC CACCTCTGAC TTGAGCGTCG ATTTTGTGA TGCTCGTCAG GGGGGCGGAG  
 4501 CCTATGGAAA AACGCCAGCA ACGCGGCCTT TTTACGGTTC CTGGCCTTTT GCTGGCCTTT  
 4561 AATCCTCATG TTCTTTCCTG CGTTATCCCC TGATTCTGTG GATAACCGTA TTACCGCCTT  
 4621 TGAGTGAGCT GATACCGCTC GCCGCAGCGG AACGACCGAG CGCAGCGAGT CAGTGAGCGA  
 4681 GGAAGCGGAA GAGCGCCTGA TGCGGTATTT TCTCCTTACG CATCTGTGCG GTATTTTACA  
 4741 CCGCATATAT GGTGCACTCT CAGTACAATC TGCTCTGATG CCGCATAGTT AAGCCAGTAT  
 4801 ACACTCCGCT ATCGTACGT GACTGGGTCA TGGCTGCGCC CCGACACCCG CCAACACCCG  
 4861 CTGACGCGCC CTGACGGGCT TGTCTGCTCC CGGCATCCGC TTACAGACAA GCTGTGACCG  
 4921 TCTCCGGGAG CTGCATGTGT CAGAGGTTTT CACCGTCATC ACCGAAACGC GCGAGGCAGC  
 4981 TGCGGTAAAG CTCATCAGCG TGGTCGTGAA GCGATTACCA GATGTCTGCC TGTTTCATCCG  
 5041 CGTCCAGCTC GTTGAGTTTC TCCAGAAGCG TTAATGTCTG GCTTCTGATA AAGCGGGCCA  
 5101 TGTTAAGGGC GGTTTTTTCC TGTTTGGTCA CTGATGCCCTC CGTGTAAGGG GGATTTCTGT  
 5161 TCATGGGGGT AATGATACCG ATGAAACGAG AGAGGATGCT CACGATACGG GTTACTGATG  
 5221 ATGAACATGC CCGGTACTG GAACGTTGTG AGGGTAAACA ACTGGCGGTA TGGATGCGGC  
 5281 GGGACCAGAG AAAAATCACT CAGGGTCAAT GCCAGCGCTT CGTTAATACA GATGTAGGTG  
 5341 TTCCACAGGG TAGCCAGCAG CATCCTGCCA TGCAGATCCG GAACATAATG GTGCAGGGCG  
 5401 CTGACTTCCG CGTTTCCAGA CTTTACGAAA CACGGAACCC GAAGACCATT CATGTTGTTG  
 5461 CTCAGGTCGC AGACGTTTTG CAGCAGCAGT CGCTTACGT TCGCTCGCGT ATCGGTGATT  
 5521 CATTCTGCTA ACCAGTAAGG CAACCCCGCC AGCCTAGCCG GGTCTCAAC GACAGGAGCA  
 5581 CGATCATGCG CACCCGTGGC CAGGACCCAA CGCTGCCCGA GATGCGCCGC GTGCGGCTGC  
 5641 TGGAGATGGC GGACGCGATG GATATGTTCT GCCAAGGGTT GGTGTGCGCA TTCACAGTTC  
 5701 TCCGCAAGAA TTGATTGGCT CCAATTCTTG GAGTGGTGAA TCCGTTAGCG AGGTGCCGCC  
 5761 GGCTTCCATT CAGGTCGAGG TGGCCCGGCT CCATGCACCG CGACGCAACG CCGGGAGGCA  
 5821 GACAAGGTAT AGGGCGGCGC CTACAATCCA TGCCAACCCG TTCCATGTGC TCGCCGAGGC  
 5881 GGCATAAATC GCCGTGACGA TCAGCGGTCC AGTGATCGAA GTTAGGCTGG TAAGAGCCGC  
 5941 GAGCGATCCT TGAAGCTGTC CCTGATGGTC GTCATCTACC TGCCTGGACA GCATGGCCTG  
 6001 CAACGCGGGC ATCCCGATGC CGCCGGAAGC GAGAAGAATC ATAATGGGGA AGGCCATCCA  
 6061 GCCTCGCGTC GCGAACGCCA GCAAGACGTA GCCCAGCGCG TCGGCCGCCA TGCCGGCGAT  
 6121 AATGGCCTGC TTCTCGCCGA AACGTTTGGT GCGGGGACCA GTGACGAAGG CTTGAGCGAG  
 6181 GCGGTGCAAG ATTCCGAATA CCGCAAGCGA CAGGCCGATC ATCGTCGCGC TCCAGCGAAA  
 6241 GCGGTCTCTG CCGAAAATGA CCCAGAGCGC TGCCGGCACC TGTCTACGA GTTGATGAT-

FIGURE 36C

94/240

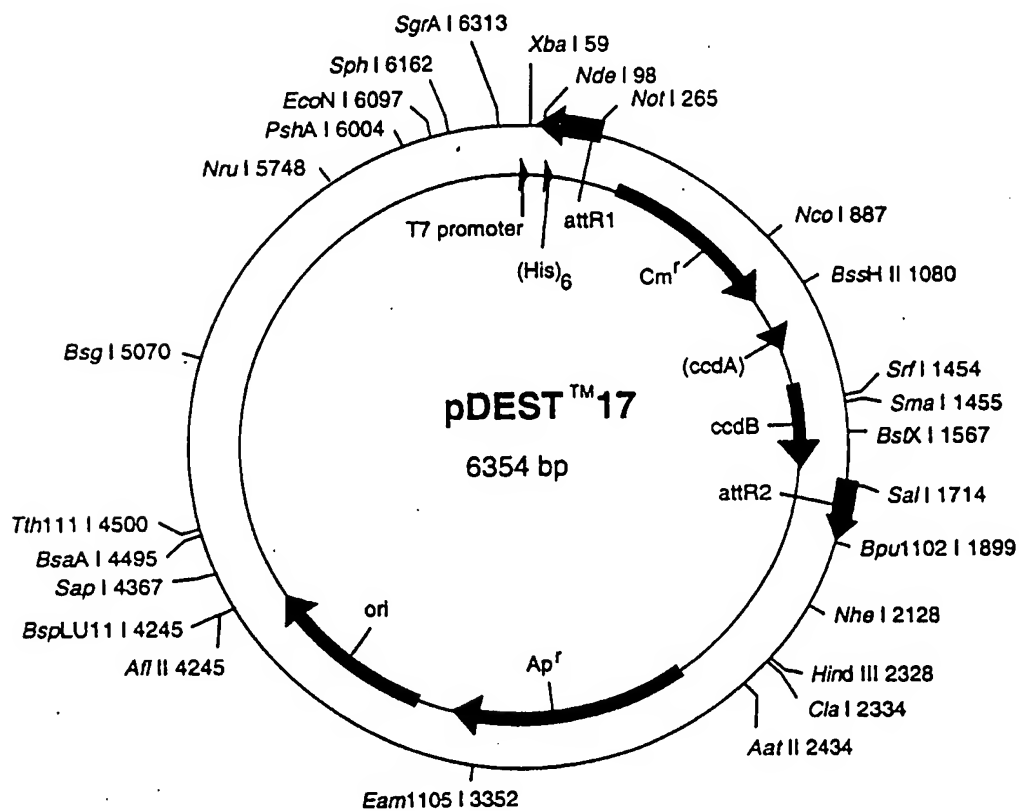
6301 AAAGAAGACA GTCATAAGTG CGGCGACGAT AGTCATGCCC CGCGCCCACC GGAAGGAGCT  
6361 GACTGGGTTG AAGGCTCTCA AGGGCATCGG TCGATCGACG CTCTCCCTTA TGCGACTCCT  
6421 GCATTAGGAA GCAGCCCAGT AGTAGGTTGA GGCCGTTGAG CACCGCCGCC GCAAGGAATG  
6481 GTGCATGCAA GGAGATGGCG CCCAACAGTC CCCC GGCCAC GGGGCCTGCC ACCATACCCA  
6541 CGCCGAAACA AGCGCTCATG AGCCCGAAGT GGCAGAGCCG ATCTTCCCCA TCGGTGATGT  
6601 CGGCGATATA GGCGCCAGCA ACCGCACCTG TGGCGCCGGT GATGCCGGCC ACGATGCGTC  
6661 CGGCGTAGAG GATCG

FIGURE 36D

95/240

1    gat ccc gcg aaa tca ata cga ctc act ata ggg aga cca caa cgg ttt ccc  
      cta ggg cgc ttt aat tat gct gag tga tat ccc tct ggt gtt gcc aaa ggg  
 52    tct aga aat aat ttt gtt taa ctt taa gaa gga gat ata cat atg tgg tac  
      aga tct tta tta aaa caa att gaa att ctt cct cta tat gta tac agc atg  
 103    Y    H    H    H    H    H    L    E    S    T    S    L    Y    K    K    A    //  
      tac cat cac cat cac cat cac ctc gaa tca aca agt ttg tac aaa aaa gct //  
      atg gta gtg gta gtg gta gtg gag ctt agt tgt tca aac atg ttt ttt cga //  
    attR1     Int Y

T7 Promoter     mRNA  
 Start Translation



## pDEST17 6354 bp

<u>Location (Base Nos.)</u>	<u>Gene Encoded</u>
258..134	attR1
367..1026	CmR
1146..1230	inactivated ccdA
1368..1673	ccdB
1714..1838	attR2
2564..3421	ampR

```

1 CGATCCCGCG AAATTAATAC GACTCACTAT AGGGAGACCA CAACGGTTTC CCTCTAGAAA
61 TAATTTTGTG TAACTTTAAG AAGGAGATAT ACATATGTCG TACTACCATC ACCATCACCA
121 TCACCTCGAA TCAACAAGTT TGTACAAAAA AGCTGAACGA GAAACGTAAA ATGATATAAA
181 TATCAATATA TTAAATTAGA TTTTGCATAA AAAACAGACT ACATAATACT GTAAAACACA
241 ACATATCCAG TCACTATGGC GGCCGCATTA GGCACCCAG GCTTTACACT TTATGCTTCC
301 GGCTCGTATA ATGTGTGGAT TTGAGTTAG GATCCGTCGA GATTTTCAGG AGCTAAGGAA
361 GCTAAAATGG AGAAAAAAT CACTGGATAT ACCACCGTTG ATATATCCCA ATGGCATCGT
421 AAAGAACATT TTGAGGCATT TCAGTCAGTT GCTCAATGTA CCTATAACCA GACCGTTCAG
481 CTGGATATTA CGGCCTTTT AAAGACCGTA AAGAAAAATA AGCACAAGTT TTATCCGGCC
541 TTTATTCACT TTCTTGCCCG CCTGATGAAT GCTCATCCGG AATTCCGTAT GGCAATGAAA
601 GACGGTGAGC TGGTGATATG GGATAGTGT CACCCTTGT ACACCGTTT CCATGAGCAA
661 ACTGAAACGT TTTTCATCGCT CTGGAGTGAA TACCACGACG ATTTCCGGCA GTTCTACAC
721 ATATATTCGC AAGATGTGGC GTGTTACGGT GAAAACCTGG CCTATTTCCC TAAAGGGTTT
781 ATTGAGAATA TGTTTTTCGT CTCAGCCAAT CCCTGGGTGA GTTTCACCAG TTTTGATTTA
841 AACGTGGCCA ATATGGACAA CTTCTTCGCC CCCGTTTTCA CCATGGGCAA ATATTATACG
901 CAAGGCGACA AGGTGCTGAT GCCGCTGGCG ATTCAGGTTT ATCATGCCGT CTGTGATGGC
961 TTCCATGTCG GCAGAATGCT TAATGAATTA CAACAGTACT GCGATGAGTG GCAGGGCGGG
1021 GCGTAAAGAT CTGGATCCGG CTTACTAAAA GCCAGATAAC AGTATGCGTA TTTGCGCGCT
1081 GATTTTTGCG GTATAAGAAT ATATACTGAT ATGTATACCC GAAGTATGTC AAAAAGAGGT
1141 GTGCTATGAA GCAGCGTATT ACAGTGACAG TTGACAGCGA CAGCTATCAG TTGCTCAAGG
1201 CATATATGAT GTCAATATCT CCGGTCTGGT AAGCACAACC ATGCAGAATG AAGCCCGTCG
1261 TCTGCGTGCC GAACGCTGGA AAGCGGAAAA TCAGGAAGGG ATGGCTGAGG TCGCCCGGTT
1321 TATTGAAATG AACGGCTCTT TTGCTGACGA GAACAGGGAC TGGTGAAATG CAGTTTAAGG
1381 TTTACACCTA TAAAAGAGAG AGCCGTTATC GTCTGTTTGT GGATGTACAG AGTGATATTA
1441 TTGACACGCC CGGGCGACGG ATGGTGATCC CCCTGGCCAG TGCACGCTG CTGTCAGATA
1501 AAGTCTCCCG TGAACCTTAC CCGGTGGTGC ATATCGGGGA TGAAGCTGCT CGCATGATGA
1561 CCACCGATAT GGCCAGTGTG CCGGTCTCCG TTATCGGGGA AGAAGTGCTT GATCTCAGCC
1621 ACCGCGAAAA TGACATCAAA AACGCCATTA ACCTGATGTT CTGGGGAATA TAAATGTCAG
1681 GCTCCCTTAT ACACAGCCAG TCTGCAGGTC GACCATAGTG ACTGGATATG TTGTGTTTTA
1741 CAGTATTATG TAGTCTGTTT TTTATGCAA ATCTAATTTA ATATATTGAT ATTTATATCA
1801 TTTTACGTTT CTCGTTTCAGC TTTCTTGAC AAAGTGGTTG ATTCGAGGCT GCTAACAAAG
1861 CCCGAAAGGA AGCTGAGTTG GCTGCTGCCA CCGCTGAGCA ATAAC TAGCA TAACCCCTTG
1921 GGGCCTCTAA ACGGGTCTTG AGGGGTTTTT TGCTGAAAGG AGGAACTATA TCCGGATATC
1981 CACAGGACGG GTGTGGTCGC CATGATCGCG TAGTCGATAG TGGCTCCAAG TAGCGAAGCG
2041 AGCAGGACTG GCGGCGGGCC AAAGCGGTCG GACAGTGCTC CGAGAACGGG TCGGCATAGA
2101 AATTGCATCA ACGCATATAG CGCTAGCAGC ACGCCATAGT GACTGGCGAT GCTGTCGGAA
2161 TGGACGATAT CCCGCAAGAG GCCCGCAGT ACCGGCATAA CCAAGCCTAT GCCTACAGCA
2221 TCCAGGTGTA CGGTGCCGAG GATGACGATG AGCGCATTGT TAGATTTTAT ACACGGTGCC
2281 TGACTGCGTT AGCAATTTAA CTGTGATAAA CTACCGCATT AAAGCTTATC GATGATAAGC
2341 TGTCAAACAT GAGAATTCTT GAAGACGAAA GGGCCTCGTG ATACGCCAT TTTTATAGGT
2401 TAATGTCATG ATAATAATGG TTTCTTAGAC GTCAAGTGCG ACTTTTCGGG GAAATGTGCG
2461 CGGAACCCCT ATTGTTTTAT TTTCTAAAT ACATTCAAAT ATGTATCCGC TCATGAGACA
2521 ATAACCCTGA TAAATGCTTC AATAATATTG AAAAAGGAAG AGTATGAGTA TTCAACATTT
2581 CCGTGTGCGC CTTATTCCCT TTTTTCGGC ATTTTGCCTT CCTGTTTTTG CTCACCCAGA
2641 AACGCTGGTG AAAGTAAAAG ATGCTGAAGA TCAGTTGGGT GCACGAGTGG GTTACATCGA-

```

FIGURE 37B

97/240

2701 ACTGGATCTC AACAGCGGTA AGATCCTTGA GAGTTTTTCGC CCCGAAGAAC GTTTTCCAAT  
 2761 GATGAGCACT TTTAAAGTTC TGCTATGTGG CGCGGTATTA TCCCGTGTG ACGCCGGGCA  
 2821 AGAGCAACTC GGTGCGCCGA TACACTATTC TCAGAATGAC TTGGTTGAGT ACTCACCAGT  
 2881 CACAGAAAAG CATCTTACGG ATGGCATGAC AGTAAGAGAA TTATGCAGTG CTGCCATAAC  
 2941 CATGAGTGAT AACACTGCGG CCAACTTACT TCTGACAACG ATCGGAGGAC CGAAGGAGCT  
 3001 AACCGCTTTT TTGCACAACA TGGGGGATCA TGTAACTCGC CTTGATCGTT GGGAAACGGGA  
 3061 GCTGAATGAA GCCATACCAA ACGACGAGCG TGACACCACG ATGCCTGCAG CAATGCGAAC  
 3121 AACGTTGCGC AAACATTATA CTGGCGAACT ACTTACTCTA GCTTCCCGGC AACAAATTAAT  
 3181 AGACTGGATG GAGGCGGATA AAGTTGCAGG ACCACTTCTG CGCTCGGCCC TTCCGGCTGG  
 3241 CTGGTTTATT GCTGATAAAT CTGGAGCCGG TGAGCGTGCG TCTCGCGGTA TCATTGCAGC  
 3301 ACTGGGGCCA GATGGTAAGC CCTCCCGTAT CGTAGTTATC TACACGACGG GGAGTCAGGC  
 3361 AACTATGGAT GAACGAAATA GACAGATCGC TGAGATAGGT GCCTCACTGA TTAAGCATTG  
 3421 GTAACGTCA GACCAAGTTT ACTCATATAT ACTTTAGATT GATTTAAAAC TTCATTTTAA  
 3481 ATTTAAAAGG ATCTAGGTGA AGATCCTTTT TGATAATCTC ATGACCAAAA TCCCTTAACG  
 3541 TGAGTTTTCG TTCCACTGAG CGTCAGACCC CGTAGAAAAG ATCAAAGGAT CTTCTTGAGA  
 3601 TCCTTTTTCG CTGCGCGTAA TCTGCTGCTT GCAAACAAA AAACCACCGC TACCAGCGGT  
 3661 GTGTTGTTTG CCGGATCAAG AGCTACCAAC TCTTTTTCG AAGGTAACGT GCATTGCAGC  
 3721 AGCGCAGATA CCAAATACTG TCCTTCTAGT GTAGCCGTAG TTAGGCCACC ACTTCAAGAA  
 3781 CTCTGTAGCA CCGCCTACAT ACCTCGCTCT GCTAATCCTG TTACCAGTGG CTGCTGCCAG  
 3841 TGGCGATAAG TCGTGTCTTA CCGGGTTGGA CTCAAGACGA TAGTTACCGG ATAAGGCGCA  
 3901 GCGGTCGGGC TGAACGGGGG GTTCGTGCAC ACAGCCCGAG TTGGAGCGAA CGACCTACAC  
 3961 CGAAGTGAAG TACCTACAGC GTGAGCTATG AGAAAGCGCC ACGCTTCCCG AAGGGAGAAA  
 4021 GCGGACAGG TATCCGGTAA GCGGCAGGGT CGGAACAGGA GAGCGCACGA GGGAGCTTCC  
 4081 AGGGGAAAC GCCTGGTATC TTTATAGTCC TGTCGGGTTT CGCCACCTCT GCACTGAGCG  
 4141 TCGATTTTGG TGATGCTCGT CAGGGGGGCG GAGCCTATGG AAAAACGCCA GCAACGCGGC  
 4201 CTTTTTACGG TTCTTGGCCT TTTGCTGGCC TTTGCTCAC ATGTTCTTTC CTGCGTTATC  
 4261 CCCTGATTCT GTGGATAACC GTATTACCGC CTTTGAGTGA GCTGATACCG CTCGCCGAG  
 4321 CCGAACGACC GAGCGCAGCG AGTCAGTGAG CGAGGAAGCG GAAGAGCGCC TGATGCGGTA  
 4381 TTTTCTCCTT ACGCATCTGT GCGGTATTTT ACACCGCATA TATGGTGCAC TCTCAGTACA  
 4441 ATCTGCTCTG ATGCCGCATA GTTAAGCCAG TATACACTCC GCTATCGCTA CGTGAAGTGG  
 4501 TCATGGCTGC GCCCGACAC CCGCCAACAC CCGCTGACGC GCCCTGACGG GCTTGTCTGC  
 4561 TCCCGGCATC CGTTACAGA CAAGCTGTGA CCGTCTCCGG GAGCTGCATG TGTCAGAGGT  
 4621 TTTCACCGTC ATCACGAAA CGCGCGAGGC AGCTGCGGTA AAGCTCATCA GCGTGGTCTG  
 4681 GAAGCGATTG ACAGATGTCT GCCTGTTTCT CCGCGTCCAG CTCGTTGAGT TTCTCCAGAA  
 4741 GCGTTAATGT CTGGCTTCTG ATAAAGCGGG CCATGTTAAG GCGGGTTTTT TCCTGTTTGG  
 4801 TCACTGATGC CTCGCTGTAA GGGGGATTTC TGTTTCATGGG GGTAAATGATA CCGATGAAAC  
 4861 GAGAGAGGAT GCTCACGATA CGGGTACTTG ATGATGAACA TGCCCGGTTA CTGGAACGTT  
 4921 GTGAGGGTAA ACAACTGGCG GTATGGATGC GCGGGGACCA GAGAAAAATC ACTCAGGGTC  
 4981 AATGCGACGC CTTGCTTAAT ACAGATGTAG GTGTTCCACA GGGTAGCCAG CAGCATCCCTG  
 5041 CGATGCAGAT CCGGAACATA ATGGTGCAGG GCGCTGACTT CCGCGTTTCC AGACTTTACG  
 5101 AAACACGGAA ACCGAAGACC ATTCATGTTG TTGCTCAGGT CGCAGACGTT TTGCAGCAGC  
 5161 AGTCGCTTCA CGTTCGCTCG CGTATCGGTG ATTCATTCTG CTAACCAGTA AGGCAACCCC  
 5221 GCCAGCTAG CCGGGTCCTC AACGACAGGA GCACGATCAT GCGCACCCGT GGCCAGGACC  
 5281 CAACGCTGCC CGAGATGCGC CGCGTGCGGC TGCTGGAGAT GGCGGACGCG ATGGATATGT  
 5341 TCTGCCAAGG GTTGGTTTGC GCATTACAG TTCTCCGCAA GAATTGATTG GCTCCAATT  
 5401 TTGGAGTGGT GAATCCGTTA GCGAGGTGCC GCCGGCTTCC ATTCAGGTG AGGTGGCCCG  
 5461 GCTCCATGCA CCGCGACGCA ACGCGGGGAG GCAGACAAGG TATAGGGCGG CGCCTACAAT  
 5521 CCATGCCAAC CCGTTCCATG TGCTCGCCGA GCGGCATATA ATCGCCGTGA CGATCAGCGG  
 5581 TCCAGTGATC GAAGTTAGGC TGGTAAGAGC CGCGAGCGAT CCTTGAAGCT GTCCCTGATG  
 5641 GTCGTATCT ACCTGCCTGG ACAGCATGGC CTGCAACGCG GGCATCCCGA TGCCCGCGGA  
 5701 AGCGAGAAGA ATCATAATGG GGAAGGCCAT CCAGCCTCGC GTCGCGAACG CCAGCAAGAC  
 5761 GTAGCCAGC GCGTCGGCCG CCATGCCGCG GATAATGGCC TGCTTCTCGC CGAAACGTTT  
 5821 GGTGGCGGGA CCACTGACGA AGGCTTGAGC GAGGGCGTGC AAGATTCCGA ATACCGCAAG  
 5881 CGACAGGCCG ATCATCGTCG CGCTCCAGCG AAAGCGGTCC TCGCCGAAAA TGACCCAGAG  
 5941 CGCTGCCGGC ACCTGTCCTA CGAGTTGCAT GATAAAGAAG ACAGTCATAA GTGCGGCGAC  
 6001 GATAGTCATG CCCCAGCGCC ACCGGAAGGA GCTGACTGGG TTGAAGGCTC TCAAGGGCAT  
 6061 CGGTCGATCG ACGCTCTCCC TTATGCGACT CCTGCATTAG GAAGCAGCCC AGTAGTAGGT  
 6121 TGAGGCCGTT GAGCACCGCC CCGCAAGGA ATGGTGCATG CAAGGAGATG GCGCCCAACA-

FIGURE 37C

98/240

6181 GTCCCCCGGC CACGGGGCCT GCCACCATAC CCACGCCGAA ACAAGCGCTC ATGAGCCCGA  
6241 AGTGGCGAGC CCGATCTTCC CCATCGGTGA TGTCGGCGAT ATAGGCGCCA GCAACCGCAC  
6301 CTGTGGCGCC GGTGATGCCG GCCACGATGC GTCCGGCGTA GAGGATCGAG ATCT

FIGURE 3ED



Figure 38A: pDEST18

FastBac Transfer Vector with p10  
Baculovirus Promoter

1 gaagacctcg gccgtcgcg gccgttgcgg tgggtgctgac cccggatgaa gtgggttcgca  
cttctggagc cggcagcgcc gcgaacggcc accacgactg gggcctactt caccaagcgt

61 tcttcgggttt tctggaaggc gagcatcggtt tgttcgcccga ggactctagc tatagttcta  
aggagccaaa agaccttccg ctcgtagcaa acaagcgggt cctgagatcg atatcaagat

121 gtgggttggtt acgtatcgag caagaaataa aaagccaaa tgcggtggag tcttctgttc  
caccaaccga tgcatacttc gttcttttat tttgcggtt gcgaacctc agaacaacg

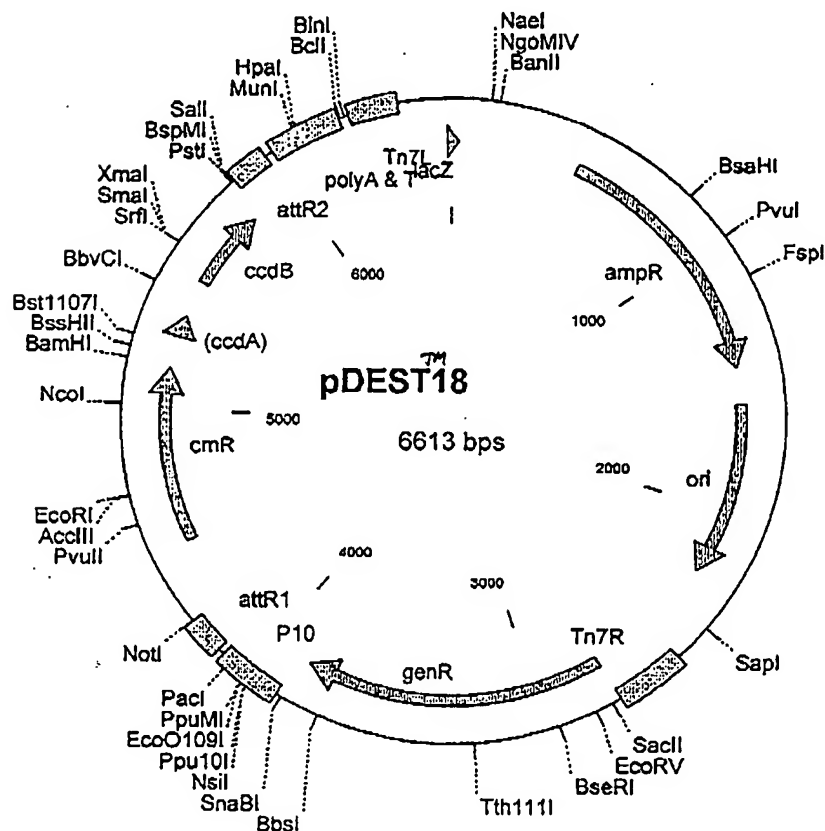
181 TTATTTTACA AAGATCAGA AATACGACG ACTTACAACA AGGGGGACTA TGAATATG  
ATAAATAAGT TTTAAAGTCT TTAAGCGTAG TGAATGTTGT TCCCCCTGAT ACTTTAATAC

241 CATTTTGAGG ATGCGGGGAC CTTTATTCAC ACCCAACACA ATATATTATA GTTAAATAG  
GTAAACTCC TACGGCCCTG GAAATTAAGT TGGTGTGTAT TATATAATAT CAATTTATCC

301 AATTATTTAT CAAATCATT GTATATTAAT TAAATACTG TACTGTAAAT TACATTTAT  
TTAATAAATA GTTTAGTAAA GATATAATTA ATTTATGAT ATGACATTAT ATGTAAATA

361 ttacaatgag gatcatcaca agtttgtaca aaaaagctga acgagaaacg taaaatgata  
aatgttactc ctagtatgt tcaaacatgt ttttcgact tgcctcttgc atttactat

...Int. attR1



100/240

## pDEST18 6613 bp

<u>Location (Base Nos.)</u>			<u>Gene Encoded</u>		
474..1449			ampR		
1590..2244			ori		
2738..3850			genR		
4251..4127			attR1		
4501..5160			CmR		
5280..5364			inactivated ccdA		
5502..5807			ccdB		
5848..5972			attR2		
6595..25			lacZ		
1	GACGCGCCCT	GTAGCGGCGC ATTAAGCGCG	GCGGGTGTGG	TGGTTACGCG	CAGCGTGACC
61	GCTACACTTG	CCAGCGCCCT AGCGCCCGCT	CCTTTCGCTT	TCTTCCCTTC	CTTTCTCGCC
121	ACGTTCCGCG	GCTTTCGCCG TCAAGCTCTA	AATCGGGGGC	TCCCTTTAGG	GTTCCGATTT
181	AGTGCTTTAC	GGCACCTCGA CCCCAGAAAA	CTTGATTAGG	GTGATGGTTC	ACGTAGTGGG
241	CCATCGCCCT	GATAGACGGT TTTTCGCCCT	TTGACGTTGG	AGTCCACGTT	CTTTAATAGT
301	GGACTCTTGT	TCCAAACTGG AACAACACTC	AACCCTATCT	CGGTCTATTC	TTTTGATTTA
361	TAAGGGATTT	TGCCGATTTC GGCCTATTGG	TTAAAAAATG	AGCTGATTTA	ACAAAAATTT
421	AACGCGAATT	TTAACAAAAT ATTAACGTTT	ACAATTTTCAG	GTGGCACTTT	TCGGGGAAAT
481	GTGCGCGGAA	CCCCTATTTG TTTATTTTTC	TAAATACATT	CAAATATGTA	TCCGCTCATG
541	AGACAATAAC	CCTGATAAAT GCTTCAATAA	TATTGAAAAA	GGAAGAGTAT	GAGTATTCAA
601	CATTTCCGTG	TCGCCCTTAT TCCCTTTTTC	GCGGCATTTT	GCCTTCCTGT	TTTTGCTCAC
661	CCAGAAACGC	TGGTGAAAGT AAAAGATGCT	GAAGATCAGT	TGGGTGCACG	AGTGGGTTAC
721	ATCGAACTGG	ATCTCAACAG CGGTAAGATC	CTTGAGAGTT	TCGCCCCGGA	AGAACGTTTT
781	CCAATGATGA	GCACTTTTAA AGTTCTGCTA	TGTGGCGCGG	TATTATCCCG	TATTGACGCC
841	GGGCAAGAGC	AACTCGGTCG CCGCATACAC	TATTCTCAGA	ATGACTTGGT	TGAGTACTCA
901	CCAGTCACAG	AAAAGCATCT TACGGATGGC	ATGACAGTAA	GAGAATTATG	CAGTGCTGCC
961	ATAACCATGA	GTGATAACAC TGC GGCCAAC	TTACTTCTGA	CAACGATCGG	AGGACCGAAG
1021	GAGCTAACCG	CTTTTGTGCA CAACATGGGG	GATCATGTAA	CTCGCCTTGA	TCGTTGGGAA
1081	CCGGAGCTGA	ATGAAGCCAT ACCAAACGAC	GAGCGTGACA	CCACGATGCC	TGTAGCAATG
1141	GCAACAACGT	TGCGCAAACT ATTAAGTGGC	GAAGTACTTA	CTCTAGCTTC	CCGGCAACAA
1201	TTAATAGACT	GGATGGAGGC GGATAAAGTT	GCAGGACCAC	TTCTGCGCTC	GGCCCTTCCG
1261	GCTGGCTGGT	TTATTGCTGA TAAATCTGGA	GCCGGTGAGC	GTGGGTCTCG	CGGTATCATT
1321	GCAGCACTGG	GGCCAGATGG TAAGCCCTCC	CGTATCGTAG	TTATCTACAC	GACGGGGAGT
1381	CAGGCAACTA	TGGATGAACG AAATAGACAG	ATCGCTGAGA	TAGGTGCCTC	ACTGATTAAAG
1441	CATTGGTAAC	TGTCAGACCA AGTTTACTCA	TATATACTTT	AGATTGATTT	AAAACCTCAT
1501	TTTTAATTTA	AAAGGATCTA GGTGAAGATC	CTTTTGTGATA	ATCTCATGAC	CAAAATCCCT
1561	TAACGTGAGT	TTTCGTTCCTA CTGAGCGTCA	GACCCCGTAG	AAAAGATCAA	AGGATCTTCT
1621	TGAGATCCTT	TTTTTCTGCG CGTAATCTGC	TGCTTGCAAA	CAAAAAAACC	ACCGCTACCA
1681	GCGGTGGTTT	GTTTGCCGGA TCAAGAGCTA	CCAACCTTTT	TTCCGAAGGT	AACTGGCTTC
1741	AGCAGAGCGC	AGATACCAAA TACTGTCTTT	CTAGTGTAGC	CGTAGTTAGG	CCACCCTTC
1801	AAGAAGCTCTG	TAGCACCGCC TACATACCTC	GCTCTGCTAA	TCCTGTTACC	AGTGGCTGCT
1861	GCCAGTGGCG	ATAAGTCGTG TCTTACCGGG	TTGGACTCAA	GACGATAGTT	ACCGGATAAG
1921	GCGCAGCGGT	CGGGCTGAAC GGGGGGTTTCG	TGCACACAGC	CCAGCTTGGA	GCGAACGACC
1981	TACACCGAAC	TGAGATACCT ACAGCGTGAG	CATTGAGAAA	GCGCCACGCT	TCCCGAAGGG
2041	AGAAAGGCGG	ACAGGTATCC GGTAAGCGGC	AGGGTCGGAA	CAGGAGAGCG	CACGAGGGAG
2101	CTTCCAGGGG	GAAACGCCTG GTATCTTTAT	AGTCCTGTCTG	GGTTTCGCCA	CCTCTGACTT
2161	GAGCGTCGAT	TTTTGTGATG CTCGTCAGGG	GGGCGGAGCC	TATGGAAAAA	CGCCAGCAAC
2221	GCGGCCTTTT	TACGGTTCCT GGCCTTTTTC	TGGCCTTTTG	CTCACATGTT	CTTTCCTGCG
2281	TTATCCCCTG	ATTCTGTGGA TAACCGTATT	ACCGCCTTTG	AGTGAGCTGA	TACCGCTCGC
2341	CGCAGCCGAA	CGACCGAGCG CAGCGAGTCA	GTGAGCGAGG	AAGCGGAAGA	GCGCCTGATG
2401	CGGTATTTTC	TCCTTACGCA TCTGTGCGGT	ATTTTCACACC	GCAGACCAGC	CGCGTAACCT
2461	GGCAAAATCG	GTTACGGTTG AGTAATAAAT	GGATGCCCTG	CGTAAGCGGG	TGTGGGCGGA-

Figure 38B

101/240

2521 CAATAAAGTC TTAAACTGAA CAAAATAGAT CTAAACTATG ACAATAAAGT CTTAAACTAG  
2581 ACAGAATAGT TGTAACACTGA AATCAGTCCA GTTATGCTGT GAAAAAGCAT ACTGGAATTT  
2641 TGTTATGGCT AAAGCAAACCT CTTTATTTTC TGAAGTGCAA ATTGCCCGTC GTATTAAAGA  
2701 GGGGCGTGCG CAAGGGCATG GTAAAGACTA TATTGCGCGC GTTGTGACAA TTTACCGAAC  
2761 AACTCCGCGG CCGGGAAGCC GATCTCGGCT TGAACGAATT GTTAGGTGGC GGTACTTGGG  
2821 TCGATATCAA AGTGCATCAC TTCTTCCCCT ATGCCCAACT TTGTATAGAG AGCCACTGCG  
2881 GGATCGTCAC CGTAATCTGC TTGCACGTAG ATCACATAAG CACCAAGCGC GTTGGCCTCA  
2941 TGCTTGAGGA GATTGATGAG CGCGGTGGCA ATGCCCTGCC TCCGGTGCTC GCCGGAZACT  
3001 GCGAGATCAT AGATATAGAT CTCACTACGC GGCTGCTCAA ACCTGGGCAG AACGTAAGCC  
3061 GCGAGAGCGC CAACAACCGC TTCTTGGTCG AAGGCAGCAA GCGCGATGAA TGTCTTACTA  
3121 CGGAGCAAAT TCCCGAGGTA ATCGGAGTCC GGCTGATGTT GGGAGTAGGT GGCTACGTCT  
3181 CCGAACTCAC GACCGAAAAG ATCAAGAGCA GCCCGCATGG ATTTGACTTG GTCAGG3CCG  
3241 AGCCTACATG TGCGAATGAT GCCCATACTT GAGCCACCTA ACTTTGTTTT AGGGCGACTG  
3301 CCCTGCTGCG TAACATCGTT GCTGCTGCGT AACATCGTTG CTGCTCCATA ACATCAAACA  
3361 TCGACCCACG GCGTAACGCG CTTGCTGCTT GGATGCCCGA GGCATAGACT GTACAAJAAA  
3421 ACAGTCATAA CAAGCCATGA AAACCGCCAC TGCGCCGTTA CCACCGCTGC GTTCGGTCAA  
3481 GGTTCCTGGAC CAGTTGCGTG AGCGCATACG CTACTTGCAAT TACAGTTTAC GAACCGAACA  
3541 GGCTTATGTC AACTGGGTTT GTGCCCTTCAT CCGTTTCCAC GGTGTGCGTC ACCCGGCAAC  
3601 CTTGGGCAGC AGCGAAGTCG AGGCATTTCT GTCCTGGCTG GCGAACGAGC GCAAGGTTTC  
3661 GGTCTCCACG CATCGTCAGG CATTGGCGGC CTTGCTGTTT TTCTACGGCA AGGTGCTGTG  
3721 CACGGATCTG CCCTGGCTTC AGGAGATCGG AAGACCTCGG CCGTCGCGGC GCTTGCCGGT  
3781 GGTGCTGACC CCGGATGAAG TGGTTCGCAT CCTCGGTTTT CTGGAAGGCG AGCATCSTTT  
3841 GTTCGCCAG GACTCTAGCT ATAGTTCTAG TGGTTGGCTA CGTATCGAGC AAGAAAATAA  
3901 AACGCCAAAC GCGTTGGAGT CTTGTGTGCT ATTTTACAA AGATTACGAA ATACGCATCA  
3961 CTTACAACAA GGGGGACTAT GAAATTATGC ATTTTGAGGA TGCCGGGACC TTTAATTCAA  
4021 CCCAACACAA TATATTATAG TTAAATAAGA ATTATTTATC AAATCATTTG TATATTAAAT  
4081 AAAATACTAT ACTGTAAATT ACATTTTATT TACAATGAGG ATCATCACAA GTTTGTACAA  
4141 AAAAGCTGAA CGAGAAACGT AAAATGATAT AAATATCAAT ATATTAAAT AGATTTCGA  
4201 TAAAAACAG ACTACATAAT ACTGTAAAC ACAACATATC CAGTCACTAT GCGGCCGCT  
4261 AAGTTGGCAG CATCACCCGA CGCACTTTGC GCCGAATAAA TACCTGTGAC GGAAGATCAC  
4321 TTCGCAGAAT AAATAAATCC TGGTGTCCCT GTTGATACCG GGAAGCCCTG GGCCAACTTT  
4381 TGGCGAAAAT GAGACGTTGA TCGGCACGTA AGAGGTTCCA ACTTTCACCA TAATGAATA  
4441 AGATCACTAC CGGGCGTATT TTTTGAGTTA TCGAGATTTT CAGGAGCTAA GGAAGCTAAA  
4501 ATGGAGAAAA AAATCACTGG ATATACCACC GTTGATATAT CCAATGGCA TCGTAAAGAA  
4561 CATTTTGAGG CATTTGATC AGTTGCTCAA TGTACCTATA ACCAGACCGT TCAGCTSGAT  
4621 ATTACGGCCT TTTTAAAGAC CGTAAAGAAA AATAAGCACA AGTTTATCC GGCCTTTATT  
4681 CACATTCCTG CCCGCTGAT GAATGCTCAT CCGGAATTCC GTATGGCAAT GAAAGACGCT  
4741 GAGCTGGTGA TATGGGATAG TGTTACCCCT TGTTACACCG TTTCCATGA GCAAACGAA  
4801 ACCTTTTTCAT CGCTCTGGAG TGAATACCAC GACGATTTCC GGCAGTTTCT ACACATATAT  
4861 TCGCAAGATG TGGCGTGTTA CCGTGAAAAC CTGGCCTATT TCCCTAAAGG GTTTATTGAG  
4921 AATATGTTTT TCGTCTCAGC CAATCCCTGG GTGAGTTTCA CCAGTTTGA TTTAAACGTC  
4981 GCCAATATGG ACAACTTCTT CGCCCCCGTT TTCACCATGG GCAAATATTA TACGCAAGGC  
5041 GACAAGGTGC TGATGCCGCT GGCGATTGAG GTTCATCATG CCGTCTGTGA TGGCTTCCAT  
5101 GTCGGCAGAA TGCTTAATGA ATTACAACAG TACTGCGATG AGTGGCAGG CGGGGC3TAA  
5161 ACGCGTGGAT CCGGCTTACT AAAAGCCAGA TAACAGTATG CGTATTTGCG CGCTGATTTT  
5221 TGCGGTATAA GAATATATAC TGATATGTAT ACCCGAAGTA TGTCAAAAG AGGTGTGCTA  
5281 TGAAGCAGCG TATTACAGTG ACAGTTGACA GCGACAGCTA TCAGTTGCTC AAGGCATATA  
5341 TGATGTCAAT ATCTCCGGTC TGTAAGCAC AACCATGCAG AATGAAGCCC GTCGCTCGCG  
5401 TGCCGAACGC TGGAAAGCGG AAAATCAGGA AGGGATGGCT GAGGTGCCCC GGTTTATTGA  
5461 AATGAACGGC TCTTTTGCTG ACGAGAACAG GGACTGGTGA AATGCAGTTT AAGGTTTACA  
5521 CCTATAAAAG AGAGAGCCGT TATCGTCTGT TTGTGGATG ACAGAGTGAT ATTATTACA  
5581 CGCCCGGGCG ACGGATGGTG ATCCCCCTGG CCAGTGACAG TCTGCTGTCA GATAAACTCT  
5641 CCCGTGAACT TTACCCGGTG GTGCATATCG GGGATGAAAG CTGGCGCATG ATGACCACCG  
5701 ATATGGCCAG TGTGCCGGTC TCCGTTATCG GGAAGAAGT GGCTGATCTC AGCCACCGCG  
5761 AAAATGACAT CAAAAACGCC ATTAACCTGA TGTCTGGGG AATATAAATG TCAGGCTCCC  
5821 TTATACACAG CCAGTCTGCA GGTGACCAT AGTGACTGGA TATGTTGTGT TTTACA3TAT  
5881 TATGTAGTCT GTTTTTTATG CAAAATCTAA TTAAATATAT TGATATTTAT ATCATTTTAC  
5941 GTTCTCTGTT CAGCTTTCTT GTACAAAGTG GTGATAGCTT GTCGAGAAGT ACTAGA3GAT-

FIGURE 38C

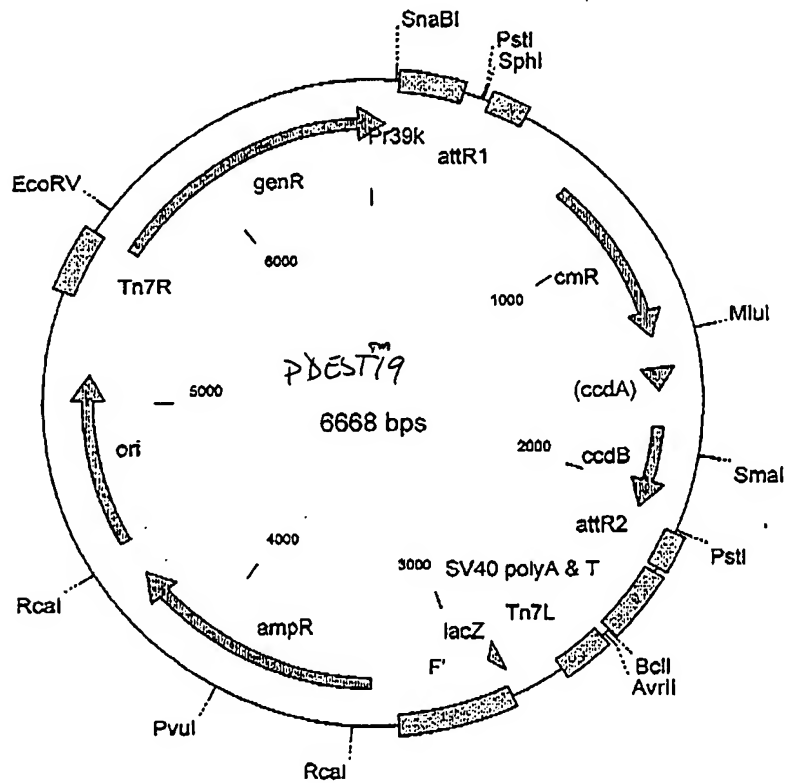
102/240

6001 CATAATCAGC CATACCACAT TTGTAGAGGT TTTACTTGCT TTAAAAAACC TCCCACACCT  
6061 CCCCCTGAAC CTGAAACATA AAATGAATGC AATTGTTGTT GTTAACTTGT TTATTGCAGC  
6121 TTATAATGGT TACAAATAAA GCAATAGCAT CACAAATTTC ACAAATAAAG CATTTTTC  
6181 ACTGCATTCT AGTTGTGGTT TGTCCAACT CATCAATGTA TCTTATCATG TCTGGATCTG  
6241 ATCACTGCTT GAGCCTAGGA GATCCGAACC AGATAAGTGA AATCTAGTTC CAAACTATTT  
6301 TGTCATTTTT AATTTTCGTA TTAGCTTACG ACGCTACACC CAGTTCCCAT CTATTTTGTC  
6361 ACTCTTCCCT AAATAATCCT TAAAACTCC ATTTCCACCC CTCCCAGTTC CCAACTATTT  
6421 TGTCCGCCCA CAGCGGGGCA TTTTCTTCC TGTATGTTT TTAATCAAAC ATCCTGCCAA  
6481 CTCCATGTGA CAAACCGTCA TCTTCGGCTA CTTTTCTCT GTACACAGAA GAAAATTTTT  
6541 CTGTCATCTC TTCGTTATTA ATGTTTGTA TTAGTGAAT ATCAACGCTT ATTTGCAGCC  
6601 TGAATGGCGA ATG

FIGURE 38D

103/240

1 ggtgacgccc tcatttttcc attgtaacgt aaatggcaac ttgtagatga acgcgctgtc  
 ccactgcccc agtagaaagg taacattgca ttaccgttg aacatctact tgcgcgacag  
 61 aaaaaaccgg ccagtttctt ccacaaactc gcgcacggct gtctcgtaaa cttttgcgtc  
 ttttttgccc ggtcaaagaa ggtgtttgag cgcgtgccga cagagcattt gaaaacgcag  
 121 // gcaacaatcg cgatgacctc gtggtatgga aattttttct aaaaaagtgt cgttcattgtc //  
 // cgttgtttagc gctactggag caccatacct ttaaaaaaga ttttttcaca gcaagtacag //  
 181 // ggccggcggc ttcgcgctcc ggtacgcgcg acgggcacac agcaggacag ccttgtccgg  
 // ccgccgccgc aagcgcgagg ccatgcgcgc tgcccgtgtg tcgtccctgc ggaacaggcc  
 241 ctcgattatc ataaacaatc ctgcaggcat gcaagctgga tcatacaag tttgtacaaa  
 gagctaatag tatttgtag gacgtccgta cgttcgacct agtaggttc aaacatgttc  
 Int V



104/240

## pDEST19 6668 bp (rotated to position 1000)

Location (Base Nos.)	Gene Encoded
515..391	attR1
765..1424	CmR
1544..1628	inactivated ccdA
1766..2071	ccdB
2112..2236	attR2
2852..2895	lacZ
3344..4319	ampR
4460..5114	ori
5608..52	genR

1	AGTGGTTTCGC	ATCCTCGGTT	TTCTGGAAGG	CGAGCATCGT	TTGTTCCGCC	AGGACTCTAG
61	CTATAGTTCT	AGTGGTTGGC	TACGTATATC	AAATACTTGT	AGGTGACGCC	GTCATCTTTC
121	CATTGTAACG	TAAATGGCAA	CTTGTAGATG	AACGCGCTGT	CAAAAAACCG	GCCAGTTTCT
181	TCCACAAACT	CGCGCACGGC	TGTCTCGTAA	ACTTTTTCGT	CGCAACAATC	GCGATGACCT
241	CGTGGTATGG	AAATTTTTC	TAAAAAGTG	TCGTTTCATG	CGGCGCGGG	CGCGTTCGCG
301	CTCCGGTACG	CGCGACGGGC	ACACAGCAGG	ACAGCCTTGT	CCGGCTCGAT	TATCATAAAC
361	AATCCTGCAG	GCATGCAAGC	TCCGATCATC	ACAAGTTTGT	ACAAAAAGC	TGAACGAGAA
421	ACGTAAATG	ATATAAATAT	CAATATATTA	AATTAGATTT	TGCATAAAAA	ACAGACTACA
481	TAATACTGTA	AAACACAACA	TATCCAGTCA	CTATGGCGGC	CGCTAAGTTG	GCAGCATCAC
541	CCGACGCACT	TTGCGCCGAA	TAAATACCTG	TGACGGAAGA	TCACTTCGCA	GAATAAATAA
601	ATCCTGGTGT	CCCTGTTGAT	ACCGGGAAGC	CCTGGGCCAA	CTTTTGGCGA	AAATGAGACG
661	TTGATCGGCA	CGTAAGAGGT	TCCAACCTTC	ACCATAATGA	AATAAGATCA	CTACCGGGCG
721	TATTTTTTGA	GTTATCGAGA	TTTTCAGGAG	CTAAGGAAGC	TAAAAATGGAG	AAAAAAATCA
781	CTGGATATAC	CACCGTTGAT	ATATCCCAAT	GGCATCGTAA	AGAACATTTT	GAGGCATTTT
841	AGTCAGTTGC	TCAATGTACC	TATAACCAGA	CCGTTTCAGT	GGATATTACG	GCCTTTTAA
901	AGACCGTAAA	GAAAAATAAG	CACAAGTTTT	ATCCGGCCTT	TATTACACAT	CTTGCCCGCC
961	TGATGAATGC	TCATCCGGAA	TTCCGTATGG	CAATGAAAGA	CGGTGAGCTG	GTGATATGGG
1021	ATAGTGTTCA	CCCTTGTTAC	ACCGTTTTCC	ATGAGCAAAC	TGAAACGTTT	TCATCGCTCT
1081	GGAGTGAATA	CCACGACGAT	TTCCGGCAGT	TTCTACACAT	ATATTCGCAA	GATGTGGCGT
1141	GTTACGGTGA	AAACCTGGCC	TATTTCCCTA	AAGGGTTTAT	TGAGAAATATG	TTTTTCGTCT
1201	CAGCCAATCC	CTGGGTGAGT	TTCACCAAGT	TTGATTTAAA	CGTGGCCAAT	ATGGACAAC
1261	TCCTCGCCCC	CGTTTTTACC	ATGGGCAAA	ATTATACGCA	AGGCGACAAG	GTGCTGATGC
1321	CGCTGGCGAT	TCAGGTTTCAT	CATGCCGTCT	GTGATGGCTT	CCATGTCCGC	AGAAATGCTTA
1381	ATGAATTACA	ACAGTACTGC	GATGAGTGGC	AGGGCGGGGC	GTAAACCGCT	GGATCCGGCT
1441	TACTAAAAGC	CAGATAACAG	TATGCGTATT	TGCGCGCTGA	TTTTTGCGGT	ATAAGAATAT
1501	ATACTGATAT	GTATACCCGA	AGTATGTCAA	AAAGAGGTGT	GCTATGAAGC	AGCGTATTAC
1561	AGTGACAGTT	GACAGCGACA	GCTATCAGTT	GCTCAAGGCA	TATATGATGT	CAATATCTCC
1621	GGTCTGGTAA	GCACAACCAT	GCAGAAATGAA	GCCCGTCGTC	TGCGTGCCGA	ACGCTGGAAA
1681	GCGGAAAATC	AGGAAGGGAT	GGCTGAGGTC	GCCCGGTTTA	TTGAAATGAA	CGGCTCTTTT
1741	GCTGACGAGA	ACAGGGACTG	GTGAAATGCA	GTTTAAGGTT	TACACCTATA	AAAGAGAGAG
1801	CCGTTATCGT	CTGTTTGTGG	ATGTACAGAG	TGATATTATT	GACACGCCCG	GGCGACGGAT
1861	GGTGATCCCC	CTGGCCAGTG	CACGTCTGCT	GTCAGATAAA	GTCTCCCGTG	AACTTTACCC
1921	GGTGGTGCAT	ATCGGGGATG	AAAGCTGGCG	CATGATGACC	ACCGATATGG	CCAGTGTGCC
1981	GGTCTCCGTT	ATCGGGGAAG	AAGTGGCTGA	TCTCAGCCAC	CGCGAAAATG	ACATCAAAAA
2041	CGCCATTAA	CTGATGTTCT	GGGGAATATA	AATGTCAGGC	TCCCTTATAC	ACAGCCAGTC
2101	TGCAGGTGCGA	CCATAGTGAC	TGGATATGTT	GTGTTTTACA	GTATTATGTA	GTCTGTTTTT
2161	TATGCAAAAT	CTAATTTAAT	ATATTGATAT	TTATATCATT	TTACGTTTCT	CGTTACGCTT
2221	TCTTGTACAA	AGTGGTGATC	GAGAAGTACT	AGAGGATCAT	AATCAGCCAT	ACCACATTTG
2281	TAGAGGTTTT	ACTTGCTTTA	AAAAACCTCC	CACACCTCCC	CCTGAACCTG	AAACATAAAA
2341	TGAATGCAAT	TGTTGTTGTT	AACCTGTTTA	TTGCAGCTTA	TAATGGTTAC	AAATAAAGCA
2401	ATAGCATCAC	AAATTTTACA	AATAAAGCAT	TTTTTTCACT	GCATTCTAGT	TGTGGTTTGT
2461	CCAAACTCAT	CAATGTATCT	TATCATGTCT	GGATCTGATC	ACTGCTTGAG	CCTAGGAGAT
2521	CCGAACCAGA	TAAGTGAAT	CTAGTTCCAA	ACTATTTTGT	CATTTTTAAT	TTTCGTATTA
2581	GCTTACGACG	CTACACCCAG	TTCCCATCTA	TTTTTGCTACT	CTTCCCTAAA	TAATCCTTAA-

FIGURE 39B

105/240

2641 AAACCTCCATT TCCACCCCTC CCAGTTCCCA ACTATTTTGT CCGCCACAG CGGGGCATT  
2701 TTCTTCCTGT TATGTTTTTA ATCAAACATC CTGCCAACTC CATGTGACAA ACCGTCATCT  
2761 TCGGCTACTT TTTCTCTGTC ACAGAATGAA AATTTTCTG TCATCTCTTC GTTATTAATG  
2821 TTTGTAATTG ACTGAATATC AACGCTTATT TGCAGCCTGA ATGGCGAATG GACGCGCCCT  
2881 GTAGCGGCGC ATTAAGCGCG GCGGGTGTGG TGGTTACGCG CAGCGTGACC GCTACACTTG  
2941 CCAGCGCCCT AGCGCCCGCT CCTTTCGCTT TCTTCCCTTC CTTCTCGCC ACGTTCGCCG  
3001 GCTTTCCTCG TCAAGCTCTA AATCGGGGCG TCCCTTTAGG GTTCCGATTT AGTGCTTTAC  
3061 GGCACCTCGA CCCCCAAAAA CTGATTAGG GTGATGGTTC ACGTAGTGGG CCATCGCCCT  
3121 GATAGACGGT TTTTCGCCCT TTGACGTTGG AGTCCACGTT CTTTAATAGT GGACTCTTGT  
3181 TCCAACTGG AACAACACTC AACCTATCT CGGTCTATTC TTTTGATTTA TAAGGGATT  
3241 TGCCGATTTC GGCTTATTGG TTAATAAATG AGCTGATTTA ACAAATTTT AACCGAATT  
3301 TTAACAAAAT ATTAACGTTT ACAATTTTCA GTGGCACTTT TCGGGGAAAT GTGCGGGAA  
3361 CCCCTATTTC TTTATTTTTT TAAATACATT CAAATATGTA TCCGCTCATG AGACAATAAC  
3421 CCTGATAAAT GCTTCAATAA TATTGAAAAA GGAAGAGTAT GAGTATTCAA CATTTCCGTG  
3481 TCGCCCTTAT TCCCTTTTTT GCGGCATTTT GCCTTCCTGT TTTTGCTCAC CCAGAAACGC  
3541 TGGTGAAAGT AAAAGATGCT GAAGATCAGT TGGGTGCACG AGTGGGTTAC ATCGAATGG  
3601 ATCTCAACAG CGGTAAGATC CTTGAGAGTT TTCGCCCCGA AGAAGCTTTT CCAATGATGA  
3661 GCACCTTTTAA AGTTCTGCTA TGTGGCGCGG TATTATCCCG TATTGACGCC GGGCAAGAGC  
3721 AACTCGGTCG CCGCATAAC TATTCTCAGA ATGACTTGGT TGAGTACTCA CCAGTCACAG  
3781 AAAAGCATCT TACGGATGGC ATGACAGTAA GAGAATTATG CAGTGCTGCC ATAACCATGA  
3841 GTGATAACAC TGCGGCCAAC TTACTTCTGA CAACGATCGG AGGACCGAAG GAGCTAACCG  
3901 CTTTTTTGCA CAACATGGGG GATCATGTAA CTCGCCTTGA TCGTTGGGAA CCGGAGCTGA  
3961 ATGAAGCCAT ACCAAACGAC GAGCGTGACA CCACGATGCC TGTAGCAATG GCAACAACGT  
4021 TGCGCAAAT ATTAAGTGGC GAACACTTTA CTCTAGCTTC CCGGCAACAA TTAATGACT  
4081 GGATGGAGGC GGATAAAGTT GCAGGACCAC TTCTGCGCTC GGCCCTTCCG GCTGGCTGGT  
4141 TTATTGCTGA TAAATCTGGA GCCGGTGAGC GTGGGTCTCG CCGTATCATT GCAGCACTGG  
4201 GGCCAGATGG TAAGCCCTCC CGTATCGTAG TTATCTACAC GACGGGGAGT CAGGCAACTA  
4261 TGGATGAACG AAATAGACAG ATCGCTGAGA TAGGTGCCCT ACTGATTAAAG CATTTGGTAAC  
4321 TGTGAGACCA AGTTTACTCA TATATACTTT AGATTGATTT AAAACTTCAT TTTTAATTTA  
4381 AAAGGATCTA GGTGAAGATC CTTTTTGATA ATCTCATGAC CAAAATCCCT TAACGTGAGT  
4441 TTTCGTTCCA CTGAGCGTCA GACCCGTTAG AAAAGATCAA AGGATCTTCT TGAGATCCTT  
4501 TTTTCTGCG CGTAATCTGC TGCTTGCAAA CAAAAAACC ACCGCTACCA GCGGTGGTTT  
4561 GTTTGCCGGA TCAAGAGCTA CCAACTCTTT TTCCGAAGGT AACTGGCTTC AGCAGAGCGC  
4621 AGATAACAAA TACTGTCTTT CTAGTGTAGC CGTAGTTAGG CCACCACTTC AAGAACTCTG  
4681 TAGCACCGCC TACATACCTC GCTCTGCTAA TCCTGTTACC AGTGGCTGCT GCCAGTGGCG  
4741 ATAAGTCGTG TCTTACCGGG TTGGACTCAA GACGATAGTT ACCGGATAAG GCGCAGCGGT  
4801 CGGGCTGAAC GGGGGGTTTC TGCACACAGC CCAGCTTGGA GCGAACGACC TACACCGAAC  
4861 TGAGATACCT ACAGCGTGAG CATTGAGAAA GCGCCACGCT TCCCGAAGGG AGAAAGCGG  
4921 ACAGGTATCC GGTAAGCGGC AGGGTCGGAA CAGGAGAGCG CACGAGGGAG CTTCCAGGGG  
4981 GAAACGCGCTG GTATCTTTAT AGTCCTGTCT GGTTCGCCA CCTCTGACTT GAGCGTCGAT  
5041 TTTTGTGATG CTCGTCAGGG GGGCGGAGCC TATGGAAAAA CGCCAGCAAC GCGGCTTTT  
5101 TACGTTTCCT GGCCTTTTGC TGGCCTTTTG CTCACATGTT CTTTCTGCG TTATCCCTG  
5161 ATTCTGTGGA TAACCGTATT ACCGCTTTTG AGTGAGCTGA TACCGCTCGC CGCAGCCGAA  
5221 CGACCGAGCG CAGCGAGTCA GTGAGCGAGG AAGCGGAAGA GCGCTGATG CGGTATTTT  
5281 TCCTTACGCA TCTGTGCGGT ATTTACACCC GCAGACCAGC CGCGTAACCT GGCAAAATCG  
5341 GTTACGGTTG AGTAATAAAT GGATGCCCTG CGTAAGCGGG TGTGGGCGGA CAATAAAGTC  
5401 TTAAACTGAA CAAAATAGAT CTAAACTATG ACAATAAAGT CTTAAACTAG ACAGAATAGT  
5461 TGTAACTGAA AATCAGTCCA GTTATGCTGT GAAAAAGCAT ACTGGACTTT TGTTATGGCT  
5521 AAAGCAAACCT CTTCAATTTT TGAAGTGCAA ATTGCCCGTC GTATTAAAGA GGGGCGTGGC  
5581 CAAGGGCATG GTAAAGACTA TATTCGCGGC GTTGTGACAA TTTACCGAAC AACTCCCGG  
5641 CCGGAAGGCC GATCTCGGCT TGAACGAATT GTTAGTGGC GGTACTTGGG TCGATATCAA  
5701 AGTGCAATCAC TTCTTCCCGT ATGCCCAACT TTGTATAGAG AGCCACTGCG GGATCGTCAC  
5761 CGTAATCTGC TTGCACGTAG ATCACATAAG CACCAAGCGC GTTGGCCTCA TGCTTGAGGA  
5821 GATTGATGAG CGCGGTGGCA ATGCCCTGCC TCCGGTGCTC GCCGGAGACT GCGAGATCAT  
5881 AGATATAGAT CTCCTACGCG GGCTGCTCAA ACCTGGGCGA AACGTAAGCC GCGAGAGCGC  
5941 CAACAACCGC TTCTTGGTCT AAGGCAGCAA GCGCGATGAA TGTCTTACTA CCGAGCAAGT  
6001 TCCCGAGGTA ATCGGAGTCC GGCTGATGTT GGGAGTAGGT GGCTACGTCT CCGAATCAC  
6061 GACCGAAAAG ATCAAGAGCA GCCCGCATGG ATTTGACTTG GTCAGGGCCG AGCCTACATG-

FIGURE 39C

106/240

6121 TCGGAATGAT GCCCATACTT GAGCCACCTA ACTTTGTTTT AGGGCGACTG CCCTGCTGCG  
6181 TAACATCGTT GCTGCTGCGT AACATCGTTG CTGCTCCATA ACATCAAACA TCGACCCACG  
6241 GCGTAACGCG CTTGCTGCTT GGATGCCCGA GGCATAGACT GTACAAAAAA ACAGTCATAA  
6301 CAAGCCATGA AAACCGCCAC TGCGCCGTTA CCACCGCTGC GTTCGGTCAA GGTTCTGGAC  
6361 CAGTTGCGTG AGCGCATACG CTACTTG CAT TACAGTTTAC GAACCGAACA GGCTTATGTC  
6421 AACTGGGTTT GTGCCTTCAT CCGTTTCCAC GGTGTGCGTC ACCCGGCAAC CTTGGGCAGC  
6481 AGCGAAGTCG AGGCATTCTT GTCCTGGCTG GCGAACGAGC GCAAGGTTTC GGTCTCCACG  
6541 CATCGTCAGG CATTGGCGGC CTTGCTGTTT TTCTACGGCA AGGTGCTGTG CACGGATCTG  
6601 CCCTGGCTTC AGGAGATCGG AAGACCTCGG CCGTCGCGGC GCTTGCCGGT GGTGCTGACC  
6661 CCGGATGA

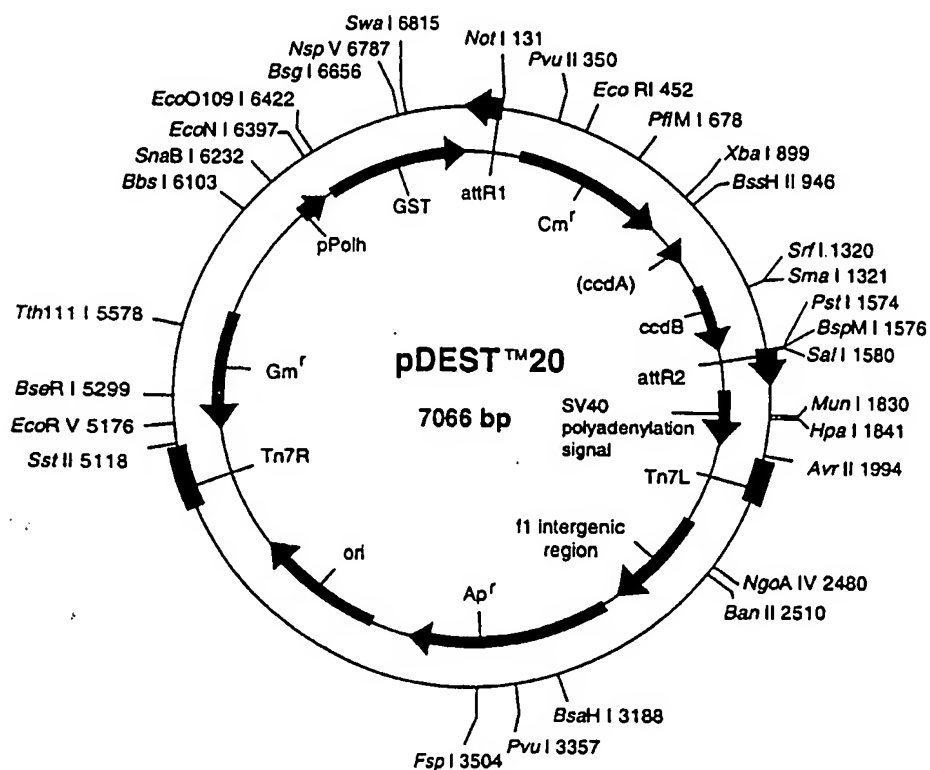
FIGURE 39A



107/240

Figure 40A: pDEST20 Glutathione-S-transferase Fusion with Polyhedron Promoter for Baculovirus Expression

430 ggc tac gta tac tcc gga ata tta ata gat cat gga gat aat taa aat gat  
 ccg atg cat atg agg cct tat aat tat cta gta cct cta tta att tta cta  
 481 aac cat ctc gca aat aaa taa gta ttt tac tgt ttt cgt aac agt ttt gta  
 ttg gta gag cgt tta ttt att cat aaa atg aca aaa gca ttg tca aaa cat  
 532 ata aaa aaa cct ata aat att cgg gat tat tca tac cgt ccc acc atc ggg  
 tat ttt ttt gga tat tta taa ggc cta ata agt atg gca ggg tgg tag ccc  
 Start Transl. → A P I - - - GST - -  
 583 cgc gga tcc atg gcc cct ata cta ggt tat tgg aaa att aag ggc ctt gtg  
 gcg cct agg tac cgg gga tat gat cca ata acc ttt taa ttc cgg gaa cac  
 S D L V P R H N Q T S L Y K R A  
 1246 tgg gat ctg gtt ccg cgt cat aat caa aca agt ttg tac aaa aaa gct gaa  
 agc cta gac caa ggc gca gta tta gtt tgt tca aac atg ttt ttt cga ctt  
 1297 cga gaa acg taa aat gat ata aat atc aat ata tta aat tag at  
 gct ctt tgc att tta cta tat tta tag tta tat aat tta atc ta



108/240

## pDEST20 7066 bp (rotated to position 5800)

<u>Location (Base Nos.)</u>			<u>Gene Encoded</u>			
	592..1263		GST			
	1397..1273		attR1			
	1506..2165		CmR			
	2285..2369		inactivated ccdA			
	2507..2812		ccdB			
	2853..2977		attR2			
	4214..5064		ampR			
	5263..5843		ori			
1	CCACTGCGCC	GTTACCACCG	CTGCGTTCGG	TCAAGGTTCT	GGACCAGTTG	CGTGAGCGCA
61	TACGCTACTT	GCATTACAGT	TTACGAACCG	AACAGGCTTA	TGTCAACTGG	GTTTCGTGCCT
121	TCATCCGTTT	CCACGGTGTG	CGTCACCCGG	CAACCTTGGG	CAGCAGCGAA	GTCGAGGCAT
181	TTCTGTCCTG	GCTGGCGAAC	GAGCGCAAGG	TTTCGGTCTC	CACGCATCGT	CAGGCATTGG
241	CGGCCTTGCT	GTTCTTCTAC	GGCAAGGTGC	TGTGCACGGA	TCTGCCCTGG	CTTCAGGAGA
301	TCGGAAGACC	TCGGCCGTCG	CGGCGCTTGC	CGGTGGTGCT	GACCCCGGAT	GAAGTGGTTC
361	GCATCCTCGG	TTTTCTGGAA	GGCGAGCATC	GTTTGTTCGC	CCAGGACTCT	AGCTATAGTT
421	CTAGTGGTTG	GCTACGTATA	CTCCGGAATA	TTAATAGATC	ATGGAGATAA	TTAAATGAT
481	AACCATCTCG	CAAATAAATA	AGTATTTTAC	TGTTTTCTGA	ACAGTTTTGT	AATAAAAAAA
541	CCTATAAATA	TTCCGGATTA	TTCATACCGT	CCCACCATCG	GGCGCGGATC	CATGGCCCCCT
601	ATACTAGGTT	ATTGGA AAAAT	TAAGGGCCTT	GTGCAACCCA	CTCGACTTCT	TTTGGAATAT
661	CTTGAAGAAA	AATATGAAGA	GCATTTGTAT	GAGCGCGATG	AAGGTGATAA	ATGGCGAAAC
721	AAAAAGTTTG	AATTGGGTTT	GGAGTTTCCC	AATCTTCCTT	ATTATATTGA	TGGTGATGTT
781	AAATTAACAC	AGTCTATGGC	CATCATACGT	TATATAGCTG	ACAAGCACAA	CATGTTGGGT
841	GGTTGTCCAA	AAGAGCGTGC	AGAGATTTCA	ATGCTTGAAG	GAGCGGTTTT	GGATATTAGA
901	TACGGTGTTT	CGAGAATTGC	ATATAGTAAA	GACTTTGAAA	CTCTCAAAGT	TGATTTTCTT
961	AGCAAGCTAC	CTGAAATGCT	GAAAATGTTC	GAAGATCGTT	TATGTCATAA	AACATATTTA
1021	AATGGTGATC	ATGTAACCCA	TCCTGACTTC	ATGTTGTATG	ACGCTCTTGA	TGTTGTTTTA
1081	TACATGGACC	CAATGTGCCT	GGATGCGTTC	CCAAAATTAG	TTTGTTTTAA	AAAACGTATT
1141	GAAGCTATCC	CACAAATTGA	TAAGTACTTG	AAATCCAGCA	AGTATATAGC	ATGGCCTTTG
1201	CAGGGCTGGC	AAGCCACGTT	TGGTGGTGGC	GACCATCCTC	CAAAATCGGA	TCTGGTTCCG
1261	CGTCATAATC	AAACAAGTTT	GTACAAAAAA	GCTGAACGAG	AAACGTAAAA	TGATATAAAT
1321	ATCAATATAT	TAAATTAGAT	TTTGCATAAA	AAACAGACTA	CATAATACTG	TAAAACACAA
1381	CATATCCAGT	CACTATGGCG	GCCGCATTAG	GCACCCAGG	CTTTACACTT	TATGCTTCCG
1441	GCTCGTATGT	TGTGTGGATT	TTGAGTTAGG	ATCCGGCGAG	ATTTTCAGGA	GCTAAGGAAG
1501	CTAAAATGGA	GAAAAAATC	ACTGGATATA	CCACCGTTGA	TATATCCCAA	TGGCATCGTA
1561	AAGAACATTT	TGAGGCATTT	CAGTCAGTTG	CTCAATGTAC	CTATAACCAG	ACCGTTCAGC
1621	TGGATATTAC	GGCCTTTTTA	AAGACCGTAA	AGAAAAATAA	GCACAAGTTT	TATCCGCGCT
1681	TTATTACAT	TCTTGCCCGC	CTGATGAATG	CTCATCCGGA	ATTCCGTATG	GCAATGAAAG
1741	ACGGTGAGCT	GGTGATATGG	GATAGTGTTT	ACCCTTGTTA	CACCGTTTTT	CATGAGCAAA
1801	CTGAAACGTT	TTCATCGCTC	TGGAGTGAAT	ACCACGACGA	TTTCCGGCAG	TTTCTACACA
1861	TATATTTCGA	AGATGTGGCG	TGTTACGGTG	AAAACCTGGC	CTATTTCCCT	AAAGGGTTTA
1921	TTGAGAATAT	GTTTTTCGTC	TCAGCCAATC	CCTGGGTGAG	TTTCACCACT	TTTGATTTAA
1981	ACGTGGCCAA	TATGGACAAC	TTCTTCGCCC	CCGTTTTTAC	CATGGGCAAA	TATTATAACG
2041	AAGGCGACAA	GGTGCTGATG	CCGCTGGCGA	TTCAGGTTCA	TCATGCCGTC	TGTGATGGCT
2101	TCCATGTCGG	CAGAATGCTT	AATGAATTAC	AACAGTACTG	CGATGAGTGG	CAGGGCGGGG
2161	CGTAATCTAG	AGGATCCGGC	TTACTAAAAG	CCAGATAACA	GTATGCGTAT	TTGCGCGCTG
2221	ATTTTTCGGG	TATAAGAATA	TATACTGATA	TGTATACCCG	AAGTATGTCA	AAAAGAGGTG
2281	TGCTATGAAG	CAGCGTATTA	CAGTGACAGT	TGACAGCGAC	AGCTATCAGT	TGCTCAAGGC
2341	ATATATGATG	TCAATATCTC	CGGTCTGGTA	AGCACAACCA	TGCAGAATGA	AGCCCGTCTG
2401	CTGCGTGCCG	AACGCTGGAA	AGCGGAAAAT	CAGGAAGGGA	TGGCTGAGGT	CGCCCGGTTT
2461	ATTGAAATGA	ACGGCTCTTT	TGCTGACGAG	AACAGGGACT	GGTGAATGTC	AGTTTAAGGT
2521	TTACACCTAT	AAAAGAGAGA	GCCGTTATCG	TCTGTTTGTG	GATGTACAGA	GTGATATTAT
2581	TGACACGCCC	GGGCGACGGA	TGGTGATCCC	CCTGGCCAGT	GCACGTCTGC	TGTCAGATAA
2641	AGTCTCCCGT	GAACCTTTACC	CGGTGGTGCA	TATCGGGGAT	GAAAGCTGGC	GCATGATGAC

Figure 40B

109/240

2701 CACCGATATG GCCAGTGTGC CGGTCTCCGT TATCGGGGAA GAAGTGGCTG ATCTCAGCCA  
2761 CCGCGAAAAT GACATCAAAA ACGCCATTAA CCTGATGTTC TGGGGAATAT AAATGTCAGG  
2821 CTCCCTTATA CACAGCCAGT CTGCAGGTCG ACCATAGTGA CTGGATATGT TGTGTTTAC  
2881 AGTATTATGT AGTCTGTTTT TTATGCAAAA TCTAATTTAA TATATTGATA TTTATATCAT  
2941 TTTACGTTTC TCGTTCAGCT TTCTTGTAACA AAGTGGTTTG ATAGCTTGTC GAGAAGTACT  
3001 AGAGGATCAT AATCAGCCAT ACCACATTTG TAGAGGTTTT ACTTGCTTTA AAAAACCTCC  
3061 CACACCTCCC CCTGAACCTG AAACATAAAA TGAATGCAAT TGTTGTGTGTT AACTTGTTTA  
3121 TTGCAGCTTA TAATGGTTAC AAATAAGCA ATAGCATCAC AAATTTTACA AATAAAGCAT  
3181 TTTTTTCACT GCATTCTAGT TGTGGTTTGT CCAAACCTCAT CAATGTATCT TATCATGTCT  
3241 GGATCTGATC ACTGCTTGAG CCTAGGAGAT CCGAACCAGA TAAGTGAAAT CTAGTCCAA  
3301 ACTATTTTGT CATTTTTAAT TTTCTGATTA GCTTACGACG CTACACCCAG TTCCCATCTA  
3361 TTTTGTCACT CTTCCCTAAA TAATCCTTAA AAACCTCCATT TCCACCCCTC CCAGTTCCCA  
3421 ACTATTTTGT CCGCCACAG CGGGGCATTT TTCTTCTGT TATGTTTTTA ATCAAACATC  
3481 CTGCCAACTC CATGTGACAA ACCGTCACTT TCGGCTACTT TTTCTCTGTC ACAGAATGAA  
3541 AATTTTTCTG TCATCTCTTC GTTATTAATG TTTGTAATTG ACTGAATATC AACGCTTATT  
3601 TGCAGCCTGA ATGGCGAATG GACGCGCCCT GTAGCGGCGC ATTAAGCGCG GCGGCTGTGG  
3661 TGGTTACGCG CAGCGTGACC GCTACACTTG CCAGCGCCCT AGCGCCCGCT CTTTCCGCTT  
3721 TCTTCCCTTC CTTTCTCGCC ACGTTCGCGG GCTTCCCGG TCAAGCTCTA AATCGGGGGC  
3781 TCCCTTTAGG GTTCCGATTT AGTGCTTTAC GGCACCTCGA CCCCCAAAAA CTTGATTAGG  
3841 GTGATGGTTC ACGTAGTGGG CCATCGCCCT GATAGACGGT TTTTCGCCCT TTGACGTTGG  
3901 AGTCCACGTT CTTTAATAGT GGACTCTTGT TCCAACTGG AACAACTC AACCCTATCT  
3961 CGGTCTATTC TTTTGATTTA TAAGGGATT TGCAGATTTC GGCCTATTGG TTAATAATG  
4021 AGCTGATTTA AAAAAAATTT AACCGCAATT TTAACAAAT ATTAACGTTT ACAATTTTCA  
4081 GTGGCACTTT TCGGGGAAAT GTGCGCGGAA CCCCTATTTG TTTATTTTTT TAAATACATT  
4141 CAAATATGTA TCCGCTCATG AGACAATAAC CCTGATAAAT GCTTCAATAA TATTGAAAAA  
4201 GGAAGAGTAT GAGTATTCAA CATTTCCGTG TCGCCCTTAT TCCCTTTTTT GCGGCATTTT  
4261 GCCTTCCGT TTTTGCTCAC CCAGAAACGC TGGTGAAAGT AAAAGATGCT GAAGATCAGT  
4321 TGGGTGCACG AGTGGGTTAC ATCGAACTGG ATCTCAACAG CGGTAAGATC CTTGAGAGTT  
4381 TTCGCCCCGA AGAACGTTTT CCAATGATGA GCACTTTTAA AGTTCTGCTA TGTGGCGCGG  
4441 TATTATCCCG TATTGACGCC GGGCAAGAGC AACTCGGTCG CCGCATACAC TATTCTCAGA  
4501 ATGACTTGGT TGAGTACTCA CCAGTCACAG AAAAGCATCT TACGGATGGC ATGACAGTAA  
4561 GAGAATTATG CAGTGCTGCC ATAACCATGA GTGATAACAC TGCGGCCAAC TTACTTCTGA  
4621 CAACGATCGG AGGACCGAAG GAGCTAACC GCTTTTTTGA CAACATGGGG GATCATGTAA  
4681 CTCGCCTTGA TCGTTGGGAA CCGGAGCTGA ATGAAGCCAT ACCAAACGAC GAGCGTGACA  
4741 CCACGATGCC TGTAGCAATG GCAACAACGT TGCGBAACT ATTAAGTGG GAACTACTTA  
4801 CTCTAGCTTC CCGGCAACAA TTAATAGACT GGATGGAGGC GGATAAAGTT GCAGGACCAC  
4861 TTCTGCGCTC GGCCCTTCCG GCTGGCTGGT TTATTGCTGA TAAATCTGGA GCCGCTGAGC  
4921 GTGGGTCTCG CCGTATCATT GCAGCACTGG GGCCAGATGG TAAGCCCTCC CGTATCGTAG  
4981 TTATCTACAC GACGGGGAGT CAGGCAACTA TGGATGAACG AAATAGACAG ATCGCTGAGA  
5041 TAGGTGCCTC ACTGATTAA GATTGGTAAC TGTCAGACCA AGTTTACTCA TATATACTTT  
5101 AGATTGATTT AAAACTTCAT TTTTAATTTA AAAGGATCTA GGTGAAGATC CTTTTTGATA  
5161 ATCTCATGAC CAAAATCCCT TAACGTGAGT TTTCGTTCCA CTGAGCGTCA GACCCCGTAG  
5221 AAAAGATCAA AGGATCTTCT TGAGATCCTT TTTTCTGCG CGTAATCTGC TGCTTGCAAA  
5281 CAAAAAACC ACCGCTACCA GCGGTGGTTT GTTTGCCGGA TCAAGAGCTA CCAACTCTTT  
5341 TTCCGAAGGT AACTGGCTTC AGCAGAGCGC AGATACCAA TACTGTCTTT CTAGTGAGC  
5401 CGTAGTTAGG CCACCACTTC AAGAACTCTG TAGCACCACC TACATACCTC GCTCTGCTAA  
5461 TCCTGTTACC AGTGGCTGCT GCCAGTGGCG ATAAGTCGTG TCTTACCGGG TTGGACTCAA  
5521 GACGATAGTT ACCGGATAAG GCGCAGCGGT CGGGCTGAAC GGGGGGTTCC TGCACACAGC  
5581 CCAGCTTGGA GCGAACGACC TACACCGAAC TGAGATACCT ACAGCGTGAG CATTGAGAAA  
5641 GCGCCACGCT TCCGAAGGG AGAAAGGCGG ACAGGTATCC GGTAAGCGGC AGGGTCGGAA  
5701 CAGGAGAGCG CACGAGGGAG CTTCCAGGGG GAAACGCCTG GTATCTTTAT AGTCCTGTCTG  
5761 GATTTTCGCCA CCTCTGACTT GAGCGTCGAT TTTTGTGATG CTCGTCAGGG GGGCGGAGCC  
5821 TATGGAAAAA CGCCAGCAAC GCGGCCTTTT TACGGTTTCT GGCCTTTTGC TGGCCTTTTG  
5881 CTCACATGTT CTTTCTGCG TTATCCCTG ATTCTGTGGA TAACCGTATT ACCGCTTTG  
5941 AGTGAGCTGA TACCGCTCG CCGCAGCGAA CGACCGAGCG CAGCGAGTCA GTGAGCGAGG  
6001 AAGCGGAAGA GCGCTGATG CCGTATTTTC TCCTTACGCA TCTGTGCGGT ATTTACACAC  
6061 GCAGACCAGC CGCGTAACCT GGCAAAATCG GTTACGGTTG AGTAATAAAT GGATGCCCTG  
6121 CGTAAGCGGG TGTGGGCGGA CAATAAAGTC TTAACCTGAA CAAAATAGAT CTAAACTATG-

FIGURE 40C

110/240

6181 ACAATAAAGT CTTAACTAG ACAGAATAGT TGTAAGTGA AATCAGTCCA GTTATGCTGT  
6241 GAAAAAGCAT ACTGGACTTT TGTTATGGCT AAAGCAAACCT CTTCAATTTTC TGAAGTGCAA  
6301 ATTGCCCCGTC GTATTAAAGA GGGGCGTGGC CAAGGGCATG GTAAAGACTA TATTCGCGGC  
6361 GTTGTGACAA TTTACCGAAC AACTCCGCGG CCGGGAAGCC GATCTCGGCT TGAACGAATT  
6421 GTTAGGTGGC GGTACTTGGG TCGATATCAA AGTGCATCAC TTCTTCCCGT ATGCCCAACT  
6481 TTGTATAGAG AGCCACTGCG GGATCGTCAC CGTAATCTGC TTGCACGTAG ATCACATAAG  
6541 CACCAAGCGC GTTGGCCTCA TGCTTGAGGA GATTGATGAG CGCGGTGGCA ATGCCCTGCC  
6601 TCCGGTGCTC GCCGGAGACT GCGAGATCAT AGATATAGAT CTCCTACGC GGCTGCTCAA  
6661 ACCTGGGCAG AACGTAAGCC GCGAGAGCGC CAACAACCGC TTCTTGGTCG AAGGCAGCAA  
6721 GCGCGATGAA TGTCTTACTA CGGAGCAAGT TCCCGAGGTA ATCGGAGTCC GGCTGATGTT  
6781 GGGAGTAGGT GGCTACGTCT CCGAACTCAC GACCGAAAAG ATCAAGAGCA GCGGCATGG  
6841 ATTTGACTTG GTCAGGGCCG AGCCTACATG TGCGAATGAT GCGCATACTT GAGCCACCTA  
6901 ACTTTGTTTT AGGGCGACTG CCCTGCTGCG TAACATCGTT GCTGCTGCGT AACATCGTTG  
6961 CTGCTCCATA ACATCAAACA TCGACCCACG GCGTAACGCG CTTGCTGCTT GGATGCCCGA  
7021 GGCATAGACT GTACAAAAA ACAGTCATAA CAAGCCATGA AAACCG

FIGURE 40D

Figure 41A:

pDEST21

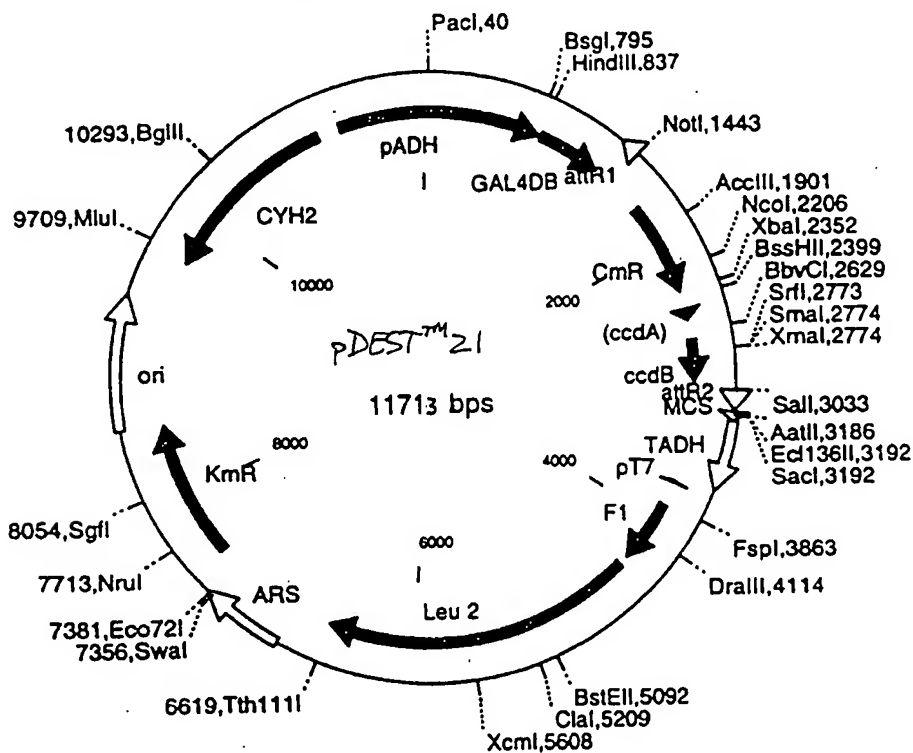
**2-Hybrid Vector with  
DNA-Binding Domain**

**ADH Promoter**

```

700  // ttg gcg ctg tgg tat caa gta taa ata gac ctg caa tta tca atc ttt tgt
    //aac ggc gaa acg ata gtt cat att tat ctg gac gtt aat aat tag aaa aca
751  // ttc ctc gtc att gtt ctc gtt ccc ttt ctt cct tgt ttc ttc ttc tgc aca
    //aag gag cag taa caa gag caa ggg aaa gaa gga aca gag ada gag acg tgt
802  // ata ttt caa gct ata cca agc ata caa tca act cca agc ttg aag caa gcc
    //tat aaa gtt cga tat ggt tgc tat gtt agt tga ggt tgc aac ttc gtt cgg
853  // tcc tga aag atg aag cta ctg tct tct atc gaa caa gca tgc gat att tgg
    //agg act ttc tac ttc gat gac aga aga tag ctt gtt cgt acg cta taa acg
...
1261 gaa gag agt agt aac aaa ggt caa aga cag ttg act gta tgc tgc agg tgc
    ctt ctc tca tca ttg ttt cca gtt tct gtc aac tga cat agc agc tcc agc
1312 aat caa aca agt ttg tac aaa aaa gct gaa cga gaa acg taa aat gat ata
    tta gtt tgt tca aac atg ttt ttt cga ctt gct ctt tgc att tta cta tat
  
```

Int + ↓



112/240

## pDEST21 11713 bp (rotated to position 11000)

<u>Location (Base Nos.)</u>	<u>Gene Encoded</u>
857..1322	GAL4DB
1456..1332	attR1
1706..2365	CmR
2485..2569	inactivated ccdA
2707..3012	ccdB
3053..3177	attR2
3716..3735	pT7 (T7 promoter)
3899..4354	f1 (f1 intergenic region)
4414..6642	Leu2
7541..8515	kanR
9668..10958	CYH2
11118..848	pADH (ADH promoter)

```

1 TTTATTATGT TACAATATGG AAGGGAAC TT TACACTTCTC CTATGCACAT ATATTAATTA
61 AAGTCCAATG CTAGTAGAGA AGGGGGGTAA CACCCCTCCG CGCTCTTTTC CGATTTTTTT
121 CTAAACCGTG GAATATTTTCG GATATCCTTT TGTGTTTCC GGGTGACAA TATGGACTTC
181 CTCTTTTCTG GCAACCAAAC CCATACATCG GGATTCCTAT AATACCTTCG TTGGTCTCCC
241 TAACATGTAG GTGGCGGAGG GGAGATATAC AATAGAACAG ATACCAGACA AGACATAATG
301 GGCTAAACAA GACTACACCA ATTACACTGC CTCATTGATG GTGGTACATA ACGAACTAAT
361 ACTGTAGCCC TAGACTTGAT AGCCATCATC ATATCGAAGT TTCACTACCC TTTTTCATT
421 TGCCATCTAT TGAAGTAATA ATAGGCGCAT GCAACTTCTT TTCTTTTTTT TTCTTTTCTC
481 TCTCCCCCGT TGTTGTCTCA CCATATCCGC AATGACAAAA AAAATGATGG AAGACACTAA
541 AGGAAAAAAT TAACGACAAA GACAGCACCA ACAGATGTCG TTGTTCCAGA GCTGATGAGG
601 GGTATCTTCG AACACACGAA ACTTTTTCCT TCCTTCATTC ACGCACACTA CTCTCTAATG
661 AGCAACGTA TACGGCCTTC CTTCAGTTA CTTGAATTTG AAATAAAAAA AGTTTGCCGC
721 TTTGCTATCA AGTATAAATA GACCTGCAAT TATTAATCTT TTGTTTCCTC GTCATTGTTT
781 TCGTTCCTCT TCTTCCTTGT TTCTTTTTCT GCACAATATT TCAAGCTATA CCAAGCATAC
841 AATCAATCC AAGCTGAAG CAAGCCTCCT GAAAGATGAA GCTACTGTCT TCTATCGAAC
901 AAGCATGCGA TATTTGCCGA CTTAAAAAGC TCAAGTGCTC CAAAGAAAAA CCGAAGTGCG
961 CCAAGTGTCT GAAGAACAAC TGGGAGTGTC GCTACTCTCC CAAAACCAA AGGTCTCCGC
1021 TGACTAGGGC ACATCTGACA GAAGTGAAT CAAGGCTAGA AAGACTGGAA CAGCTATTTT
1081 TACTGATTTT TCCTCGAGAA GACCTTGACA TGATTTTGAA AATGGATTCT TTACAGGATA
1141 TAAAGCATT GTTAACAGGA TTATTTGTAC AAGATAATGT GAATAAAGAT GCCGTCACAG
1201 ATAGATTGGC TTCAGTGGAG ACTGATATGC CTCTAACATT GAGACAGCAT AGAATAAGTG
1261 CGACATCATC ATCGGAAGAG AGTAGTAACA AAGGTCAAAG ACAGTTGACT GTATCGTCGA
1321 GGTCGAATCA AACAAGTTTG TACAAAAAAG CTGAACGAGA AACGTAAAT GATATAAATA
1381 TCAATATATT AAATTAGATT TTGCATAAAA AACAGACTAC ATAATACTGT AAAACACAAC
1441 ATATCCAGTC ACTATGGCGG CCGCTAAGTT GGCAGCATCA CCCGACGCAC TTTGCGCCGA
1501 ATAAATACCT GTGACGGAAG ATCACTTCGC AGAATAAATA AATCCTGGTG TCCCTGTTGA
1561 TACCGGAAG CCCTGGGCCA ACTTTTGGCG AAAATGAGAC GTTGATCGGC ACGTAAGAGG
1621 TTCCAAC TTTT CACCATAATG AAATAAGATC ACTACCGGGC GTATTTTTTG AGTTATCGAG
1681 ATTTTCAGGA GCTAAGGAAG CTAAAATGGA GAAAAAATC ACTGGATATA CCACCGTTGA
1741 TATATCCCAA TGGCATCGTA AAGAACATTT TGAGGCATTT CAGTCAGTTG CTCAATGTAC
1801 CTATAACCAG ACCGTTTCAGC TGGATATTAC GGCTTTTTTA AAGACCGTAA AGAAAAATAA
1861 GCACAAGTTT TATCCGGCCT TTATTACAT TCTTGCCCGC CTGATGAATG CTCATCCGGA
1921 ATTCCGTATG GCAATGAAAG ACGGTGAGCT GGTGATATGG GATAGTGTTC ACCCTTGTTA
1981 CACCGTTTTT CATGAGCAAA CTGAAACGTT TTCATCGCTC TGGAGTGAAT ACCACGACGA
2041 TTTCCGGCAG TTTCTACACA TATATTGCGA AGATGTGGCG TGTTACGGTG AAAACCTGGC
2101 CTATTTCCCT AAAGGGTTTA TTGAGAATAT GTTTTTCGTC TCAGCCAATC CCTGGGTGAG
2161 TTTCACCACT TTTGATTTAA ACGTGGCCAA TATGGACAAC TTCTTCGCCC CCGTTTTTAC
2221 CATGGGCAAA TATTATACGC AAGGCGACAA GGTGCTGATG CCGCTGGCGA TTCAGGTTCA
2281 TCATGCCGTC TGTGATGGCT TCCATGTCGG CAGAAATGCTT AATGAATTAC AACAGTACTG
2341 CGATGAGTGG CAGGGCGGGG CGTAATCTAG AGGATCCGGC TTAATAAAG CCAGATAACA
2401 GTATGCGTAT TTGCGCGCTG ATTTTTCGGG TATAAGAATA TATACTGATA TGTATACCGG-

```

FIGURE 413

```

2461 AAGTATGTCA AAAAGAGGTG TGCTATGAAG CAGCGTATTA CAGTGACAGT TGACAGCGAC
2521 AGCTATCAGT TGCTCAAGGC ATATATGATG TCAATATCTC CGGTCTGGTA AGCACAACCA
2581 TGCAGAAATGA AGCCCGTCGT CTGCGTGCCG AACCGTGGAA AGCGGAAAAT CAGGAAGGGA
2641 TGGCTGAGGT CGCCCGGTTT ATTGAAATGA ACGGCTCTTT TGCTGACGAG AACAGGGACT
2701 GGTGAAATGC AGTTTAAAGT TTACACCTAT AAAAGAGAGA GCCGTTATCG TCTGTTTGTG
2761 GATGTACAGA GTGATATTAT TGACACGCCC GGGCGACGGA TGGTGATCCC CCTGGCCAGT
2821 GCACGTCTGC TGTCAGATAA AGTCTCCCGT GAACTTTACC CGGTGGTGCA TATCGGGGAT
2881 GAAAGCTGGC GCATGATGAC CACCGATATG GCCAGTGTGC CGGTCTCCGT TATCGGGGAA
2941 GAAGTGGCTG ATCTCAGCCA CCGCGAAAAT GACATCAAAA ACGCCATTAA CCTGATGTTT
3001 TGGGGAATAT AAATGTCAGG CTCCTTATA CACAGCCAGT CTGCAGGTG ACCATAGTGA
3061 CTGGATATGT TGTGTTTAC AGTATTATGT AGTCTGTTT TTATGCAAAA TCTAATTTAA
3121 TATATTGATA TTTATATCAT TTTACGTTTC TCGTTCAGCT TTCTGTACA AAGTGGTTTG
3181 ATGGCCGCTA AGTAAGTAAG ACGTCGAGCT CTAAGTAAGT AACGCCGCC ACCGCGGTGG
3241 AGCTTTGGAC TTCTTCGCCA GAGGTTTGGT CAAGTCTCCA ATCAAGGTTG TCGGCTTGTC
3301 TACCTTGCCA GAAATTTACG AAAAGATGGA AAAGGGTCAA ATCGTTGGTA GATACGTTGT
3361 TGACACTTCT AAATAAGCGA ATTTCTTATG ATTTATGATT TTTATTATTA AATAAGTTAT
3421 AAAAAAATA AGTGATACA AATTTTAAAG TGAATCTTAG GTTTTAAAC AAAAATCTT
3481 ATTCTTGAGT AACTCTTCC TGTAGGTCAG GTTGCTTTCT CAGGTATAGC ATGAGGTCGC
3541 TCTTATTGAC CACACCTCTA CCGGCATGCC GAGCAAATGC CTGCAAATCG CTCCCCATTT
3601 CACCCAATTG TAGATATGCT AACTCCAGCA ATGAGTTGAT GAATCTCGGT GTGTATTTTA
3661 TGTCCTCAGA GGACAATACC TGTGTAATC GTTCTTCCAC ACGGATCCCA ATTCGCCCTA
3721 TAGTGAGTCG TATTACAATT CACTGGCCGT CGTTTTACAA CGTCGTGACT GGGAAAACCC
3781 TGGCGTTACC CAACTTAATC GCCTTGACG ACATCCCCCT TTCGCCAGT GCGTAATAG
3841 CGAAGAGGCC CGCACCAGT CCGCTTCCCA ACAGTTGCCG AGCCTGAATG CGGAATGGAC
3901 GCGCCCTGTA GCGGCGCATT AAGCGCGGCG GGTGTGGTGG TTACGCGCAG CGTGACCGCT
3961 ACACTTGCCA GCGCCCTAGC GCCCGCTCCT TTCGCTTTCT TCCCTTCCTT TCTCGCCACG
4021 TTCGCCGCT TTCGCCGCTA AGCTCTAAAT CGGGGGCTCC CTTTAGGGT CCGATTTAGT
4081 GCTTTACGGC ACCTCGACCC CAAAAAATT GATTAGGGTG ATGGTTCACG TAGTGGGCCA
4141 TCGCCCTGAT AGACGGTTTT TCGCCCTTG ACGTTGGAGT CCACGTTCTT TAATAGTGGA
4201 CTCTTGTTCC AAAGTGAAC AACACTCAAC CCTATCTCG TCTATTCTTT TGATTTATAA
4261 GGGATTTTGC CGATTTTCGC CTATTGGTTA AAAAAATGAG TGATTTAACA AAAATTTAAC
4321 GCGAATTTTA ACAAATATT AACGTTTACA ATTTCTGAT GCGGTATTTT CTCCTTACGC
4381 ATCTGTGCGG TATTTACAC CGCATATCGA CCGGTCGAGG AGAACTCTA GTATATCCAC
4441 ATACCTAATA TTATTGCCTT ATTAAAAATG GAATCGGAAC AATTACATCA AAATCCACAT
4501 TCTCTTCAA ATCAATTGTC CTGTACTTCC TTGTTTCATGT GTGTTCAAAA ACGTTATATT
4561 TATAGGATAA TTATACTCTA TTTCTCAACA AGTAATTGGT TGTTTGCCG AGCGGTCTAA
4621 GCGCCTGAT TCAAGAAATA TCTTGACCG AGTTAACTGT GGAATACTC AGGTATCGTA
4681 AGATGCAAGA GTTCGAATCT CTTAGCAACC ATTATTTTTT TCCTCAACAT AACGAGAACA
4741 CACAGGGGCG CTATCGCACA GAATCAAATT CGATGACTGG AAATTTTTTG TTAATTTTCA
4801 AGGTGCGCTG ACGCATATAC CTTTTTCAAC TGAAAAATTG GGAGAAAAAG GAAAGGTGAG
4861 AGGCCGGAAC CGGCTTTTCA TATAGAATAG AGAAGCGTTC ATGACTAAAT GCTTGCATCA
4921 CAATACTTGA AGTTGACAAT ATTATTTAAG GACCTATTGT TTTTCCAAT AGGTGGTTAG
4981 CAATCGTCTT ACTTTCTAAC TTTTCTTACC TTTTACATTT CAGCAATATA TATATATATT
5041 TCAAGGATAT ACCATTCTAA TGTCTGCCC TATGTCTGCC CTAAGAAGA TCGTCGTTTT
5101 GCCAGGTGAC CACGTTGGTC AAGAAATCAC AGCCGAAGCC ATTAAGGTTT TTAAGCTAT
5161 TTCTGATGTT CGTTCCAATG TCAAGTTCGA TTTGAAAAT CATTTAATTG GTGGTGCTGC
5221 TATCGATGCT ACAGGTGTCC CACTTCCAGA TGAGGCGCTG GAAGCCTCCA AGAAGGTGA
5281 TGCCGTTTTG TTAGGTGCTG TGGGTGGTCC TAAATGGGGT ACCGGTAGTG TTAGACCTGA
5341 ACAAGGTTTA CTAAAAATCC GTAAAGAACT TCAATTGTAC GCCAACTTAA GACCATGTAA
5401 CTTTGTCATCC GACTCTCTTT TAGACTTATC TCCAATCAAG CCACAATTTG CTAAGGTAC
5461 TGACTTCGTT GTTGTCAGAG AATTAGTGGG AGGTATTTAC TTTGGTAAGA GAAAGGAAGA
5521 CGATGGTGAT GGTGTCGCTT GGGATAGTGA ACAATACACC GTTCCAGAAG TGCAAAGAAT
5581 CACAAGAATG GCCGCTTTCA TGGCCCTACA ACATGAGCCA CCATTGCCTA TTTGGTCTT
5641 GGATAAAGCT AATGTTTTGG CCTCTTCAAG ATTATGGAGA AAAACTGTGG AGGAAACCAT
5701 CAAGAACGAA TTCCCTACAT TGAAGGTTCA ACATCAATTG ATTGATTCTG CCGCCATGAT
5761 CCTAGTTAAG AACCACACCC ACCTAAATGG TATTATAATC ACCAGCAACA TGTTTGGTGA
5821 TATCATCTCC GATGAAGCCT CCGTTATCCC AGGTTCCCTG GGTGTTGTGC CATCTGCGTC
5881 CTTGGCCTCT TTGCCAGACA AGAACACCGC ATTTGGTTTG TACGAACCAT GCCACGGTTC-

```

Figure 41C

5941 TGCTCCAGAT TTGCCAAAGA ATAAGGTTGA CCCTATCGCC ACTATCTTGT CTGCTGCAAT  
6001 GATGTTGAAA TTGTCATTGA ACTTGCCTGA AGAAGGTAAG GCCATTGAAG ATGCASTTAA  
6061 AAAGGTTTTG GATGCAGGTA TCAGAAGTGG TGATTTAGGT GGTTCACACA GTACCACCGA  
6121 AGTCGGTGAT GCTGTCGCCG AAGAAGTTAA GAAAATCCTT GCTTAAAAAG ATTCTCTTTT  
6181 TTTATGATAT TTGTACATAA ACTTTATAAA TGAAATTCAT AATAGAAACG ACACGAAATT  
6241 ACAAATGGA ATATGTTTCAT AGGGTAGACG AAATATATA CGCAATCTAC ATACATTTAT  
6301 CAAGAAGGAG AAAAAGGAGG ATAGTAAAGG AATACAGGTA AGCAAATTGA TACTAATGGC  
6361 TCAACGTGAT AAGGAAAAAG AATTGCACCT TAACATTAAT ATTGACAAGG AGGAGGGCAC  
6421 CACACAAAAA GTTAGGTGTA ACAGAAAATC ATGAAACTAC GATTCCTAAT TTGATATTGG  
6481 AGGATTTTCT CTAAAAAAA AAAAATACAA CAAATAAAAA AACTCAATG ACCTGACCAT  
6541 TTGATGGAGT TTAAGTCAAT ACCTTCTTGA ACCATTTCCC ATAATGGTGA AAGTTCCCTC  
6601 AAGAATTTTA CTCTGTCAGA AACGGCCTTA CGACGTAGTC GATATGGTGC ACTCTCAGTA  
6661 CAATCTGCTC TGATGCCGCA TAGTTAAGCC AGCCCCGACA CCGCCAACA CCGCTGACG  
6721 CGCCCTGACG GGCTTGCTCTG CTCCCGGCAT CCGCTTACAG ACAAGCTGTG ACCGTCTCCG  
6781 GGACGTGCAT GTGTCAGAGG TTTTCACCGT CATCACCGAA ACGCGCGAGA CGAAAGGGCC  
6841 TCGTGATACG CCTATTTTGA TAGGTTAATG TCATGATAAT AATGGTTTCT TAGGACGGAT  
6901 CGCTTGCCCTG TAACTTACAC GCGCCTCGTA TCTTTTAAATG ATGGAATAAT TTGGGAATTT  
6961 ACTCTGTGTT TATTTATTTT TATGTTTGT ATTTGGATTT TAGAAAAGTAA ATAAAGAAGG  
7021 TAGAAGAGTT ACGGAATGAA GAAAAAATAA TAAACAAAGG TTTAAAAAAT TTCAACAAAA  
7081 AGCGTACTTT ACATATATAT TTATTAGACA AGAAAAGCAG ATTAATAAGA TATACATTCTG  
7141 ATTAACGATA AGTAAATGT AAAATCACAG GATTTTCGTG TGTGGTCTTC TACACAGACA  
7201 AGATGAAACA ATTCGGCATT AATACCTGAG AGCAGGAAGA GCAAGATAAA AGGTAGTATT  
7261 TGTTGGCGAT CCCCCTAGAG TCTTTTACAT CTTCCGAAAA CAAAAACTAT TTTTCTTTA  
7321 ATTTCTTTTT TTAATTTCTA TTTTAAATTT ATATATTTAT ATTAAAAAAT TTAAATTATA  
7381 ATTATTTTTA TAGCACGTGA TGAAAAGGAC CCAGGTGGCA CTTTTCGGGG AAATGTGCGC  
7441 GGAACCCCTA TTTGTTTATT TTTCTAAATA CATTCAAATA TGTATCCGCT CATGAGACAA  
7501 TAACCCTGAT AAATGCTTCA ATAATCTGCA GCTCTGGCCC GTGTCTCAA ATCTCTGATG  
7561 TTACATTGCA CAAGATAAAA ATATATCATC ATGAACAATA AACTGTCTG CTTACATAAA  
7621 CAGTAATACA AGGGGTGTTA TGAGCCATAT TCAACGGGAA ACGTCTTGCT GGAGGCCCGG  
7681 ATTAAATCC AACATGGATG CTGATTTATA TGGGTATAAA TGGGCTCGCG ATAATGTCCG  
7741 GCAATCAGGT GCGACAATCT TTCGATTGTA TGGGAAGCCC GATGCGCCAG AGTTGTTTCT  
7801 GAAACATGGC AAAGGTAGCG TTGCCAATGA TGTTACAGAT GAGATGGTCA GACTAAACTG  
7861 GCTGACGGAA TTTATGCCTC TTCCGACCAT CAAGCATTTT ATCCGTACTC CTGATGATGC  
7921 ATGGTTACTC ACCACTGCGA TCCGCGGGAA AACAGCATTC CAGGTATTAG AAGAATATCC  
7981 TGATTCAGGT GAAAATATTG TTGATGCGCT GGCAGTGTTC CTGCGCCGGT TGCATTCGAT  
8041 TCCTGTTTGT AATTGTCTTT TTAACAGCGA TCGCGTATTT CGTCTCGCTC AGGCGCAATC  
8101 ACGAATGAAT AACGGTTTGG TTGATGCGAG TGATTTTGAT GACGAGCGTA ATGGCTGGCC  
8161 TGTTGAACAA GTCTGGAAG AAATGCATAC GCTTTTGCCA TTCTCACCGG ATTCAGTCGT  
8221 CACTCATGGT GATTTCTCAC TTGATAACCT TATTTTGTAC GAGGGGAAAT TAATAGGTTG  
8281 TATTGATGTT GGACGAGTCG GAATCGCAGA CCGATACCAG GATCTTGCCA TCCTATGGAA  
8341 CTGCCTCGGT GAGTTTCTC CTTCATTACA GAAACGGCTT TTTCAAAAAT ATGGTATTGA  
8401 TAATCCTGAT ATGAATAAAT TGCAGTTTCA TTTGATGCTC GATGAGTTTT TCTAATCAGA  
8461 ATTGGTTAAT TGGTTGTAAC ACTGGCAGAG CATTACGCTG ACTTGACGGG ACGGCGCATG  
8521 ACCAAAATCC CTTAACGTGA GTTTTCGTTC CACTGAGCGT CAGACCCCGT AGAAAAGATC  
8581 AAAGGATCTT CTTGAGATCC TTTTTTCTG CGCGTAATCT GCTGCTTGCA AACAAAAAA  
8641 CCACCGCTAC CAGCGGTGGT TTGTTTGCCG GATCAAGAGC TACCAACTCT TTTTCCGAAG  
8701 GTAACGGCT TCAGCAGAGC GCAGATACCA AATACTGTCC TTCTAGTGTA GCCGTAGTTA  
8761 GGCCACCACT TCAAGAACTC TGTAGCACC CTACATACC TCGCTCTGCT AATCCTGTTA  
8821 CCAGTGCTG CTGCCAGTGG CGATAAGTCG TGTCTTACCG GGTGGACTC AAGACGATAG  
8881 TTACCGGATA AGGCGCAGCG GTCGGGTGTA ACGGGGGGTT CGTGACACA GCCCAGCTTG  
8941 GAGCGAACGA CCTACACCGA ACTGAGATAC CTACAGCGTG AGCATTGAGA AAGCGCCACG  
9001 CTTCCCGAAG GGAGAAAGGC GGACAGGTAT CCGGTAAGCG GCAGGGTCGG AACAGGAGAG  
9061 CGCAGGAGGG AGCTTCCAGG GGGGAACGCC TGGTATCTTT ATAGTCCTGT CGGGTTTCGC  
9121 AACCTCTGAC TTGAGCGTCG ATTTTGTGTA TGCTCGTCAG GGGGGCCGAG CCTATGGAAA  
9181 CACGCCAGCA ACGCGGCCTT TTTACGGTTC CTGGCCTTTT GCTGGCCTTT TGCTCACATG  
9241 TTCTTTCTCG CGTTATCCCC TGATTCTGTG GATAACCGTA TTACCGCCTT TGAGTGAGCT  
9301 GATACCGCTC GCCGCGAGCG AACGACCGAG CGCAGCGAGT CAGTGAGCGA GGAAGCGGAA  
9361 GAGCGCCCAA TACGCAAACC GCCTCTCCCC GCGCGTTGGC CGATTCATTA ATGCAGCTGG-

FIGURE 4D



115/240

9421 CACGACAGGT TTCCCGACTG GAAAGCGGGC AGTGAGCGCA ACGCAATTAA TGTGAGTTAC  
9481 CTCACCTCATT AGGCACCCCA GGCTTTACAC TTTATGCTTC CGGCTCCTAT GTTGTGTGGA  
9541 ATTGTGAGCG GATAACAATT TCACACAGGA AACAGCTATG ACCATGATTA CGCCAAGCTC  
9601 GGAATTAACC CTCACTAAAG GGAACAAAAG CTGGTACCGA TCCCGAGCTT TGCAAATTAA  
9661 AGCCTTCGAG CGTCCCAAAA CCTTCTCAAG CAAGGTTTTT AGTATAATGT TACATGCGTA  
9721 CACGCGTCTG TACAGAAAAA AAAGAAAAAT TTGAAATATA AATAACGTTT TTAATACTAA  
9781 CATAACTATA AAAAAATAAA TAGGGACCTA GACTTCAGGT TGTCTAACTC CTTCCCTTTT  
9841 GGTTAGAGCG GATGTGGGGG GAGGGCGTGA ATGTAAGCGT GACATAACTA ATTACATGAT  
9901 ATCGACAAAG GAAAAGGGGC CTGTTTACTC ACAGGCTTTT TTCAAGTAGG TAATTAAGTC  
9961 GTTTCTGTCT TTTTCTTCT TCAACCCACC AAAGGCCATC TTGGTACTTT TTTTTTTTTT  
10021 TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT  
10081 TTTTTTTTTT TTTTTTTTTT TCATAGAAAT AATACAGAAG TAGATGTTGA ATTAGATTAA  
10141 ACTGAAGATA TATAATTTAT TGGAAATAC ATAGAGCTTT TTGTTGATGC GCTTAAGCGA  
10201 TCAATTCAAC AACACCACCA GCAGCTCTGA TTTTTCTTC AGCCAACCTG GAGACGAATC  
10261 TAGCTTTGAC GATAACTGGA ACATTTGGA TTCTACCCTT ACCCAAGATC TTACCGTAAC  
10321 CGGCTGCCAA AGTGTCATA ACTGGAGCAG TTTCTTAGA AGCAGATTTC AAGTATTGGT  
10381 CTCTCTGTG TTTCTGGATC AATGTCCACA ATTTGTCCAA GTTCAAGACT GGCTTCCAGA  
10441 AATGAGCTTG TTGCTTGTGG AAGTATCTCA TACCAACCTT ACCGAAATAA CCTGGATGGT  
10501 ATTTATCCAT GTTAATTCTG TGGTGATGTT GACCACCGGC CATACCTCTA CCACCGGGGT  
10561 GCTTTCTGTG CTTACCGATA CGACCTTTAC CGGCTGAGAC GTGACCTCTG TGCTTTCTAG  
10621 TCTTAGTGAA TCTGGAAGGC ATTCTTGATT AGTTGGATGA TTGTTCTGGG ATTTAATGCA  
10681 AAAATCACTT AAGAAGGAAA ATCAACGGAG AAAGCAAACG CCATCTTAAA TATACGGGAT  
10741 ACAGATGAAA GGGTTTGAAC CTATCTGGAA AATAGCATT AACAAGCGAA AAACCTCGAG  
10801 GAAAAATTGT TGCGTCTCTG CGGGCTATTC ACGCGCCAGA GGAAAATAGG AAAAATAACA  
10861 GGGCATTAGA AAAATAATTT TGATTTTGGT AATGTGTGGG TCCTGGTGTA CAGATGTTAC  
10921 ATTGTTTACA GTACTCTTGT TTTTGCTGTG TTTTTCGATG AATCTCCAAA ATGGTTGTTA  
10981 GCACATGGAA GAGTCACCGA TGCTAAGTTA TCTCTATGTA AGCTACGTGG CGTGACTTTT  
11041 GATGAAGCCG CACAAGAGAT ACAGGATTGG CAACTGCAAA TAGAATCTGG GGATCCCCC  
11101 TCGAGATCCG GGATCGAAGA AATGATGGTA AATGAAATAG GAAATCAAGG AGCATGAAGG  
11161 CAAAAGACAA ATATAAGGGT CGAACGAAAA ATAAAGTGAA AAGTGTTGAT ATGATGTATT  
11221 TGGCTTTGCG GCGCCGAAAA AACGAGTTTA CGCAATTGCA CAATCATGCT GACTCTGTGG  
11281 CGGACCCGCG CTCTTGCCGG CCCGGCGATA ACGCTGGGCG TGAGGCTGTG CCCGGCGGAG  
11341 TTTTTTGCGC CTGCATTTTC CAAGGTTTAC CCTGCGCTAA GGGGCGAGAT TGGAGAAGCA  
11401 ATAAGAATGC CGGTTGGGGT TGCGATGATG ACGACCACGA CAACTGGTGT CATTATTTAA  
11461 GTTGCCGAAA GAACCTGAGT GCATTTGCAA CATGAGTATA CTAGAAGAAT GAGCCAAGAC  
11521 TTGCGAGACG CGAGTTTGCC GGTGGTGCGA ACAATAGAGC GACCATGACC TTGAAGGTGA  
11581 GACGCGCATA ACCGCTAGAG TACTTTGAAG AGGAAACAGC AATAGGGTTG CTACCAATAT  
11641 AAATAGACAG GTACATACAA CACTGGAAAT GGTGTCTGT TTGAGTACGC TTTCAATTCA  
11701 TTTGGGTGTG CAC

FIGURE 41E

Figure 42A:

pDEST22

2-Hybrid Vector with  
Activation Domain

657 acg cac act act ctc taa tga gca acg gta tac ggc ctt cct tcc agt tac  
tgc gtg tga tga gag att act cgt tgc cat atg ccg gaa gga agg tca atg

708 ttg aat ttg aaa taa aaa aag ttt gcc gct ttg cta tca agt ata aat aga  
aac tta aac ttt att ttt ttc aaa cgg cga aac gat agt tca tat tta tct

759 cct gca att att aat ctt ttg ttt cct cgt cat tgt tct cgt tcc ctt tct  
gga cgt taa taa tta gaa aac aaa gga gca gta aca aga gca agg gaa aga

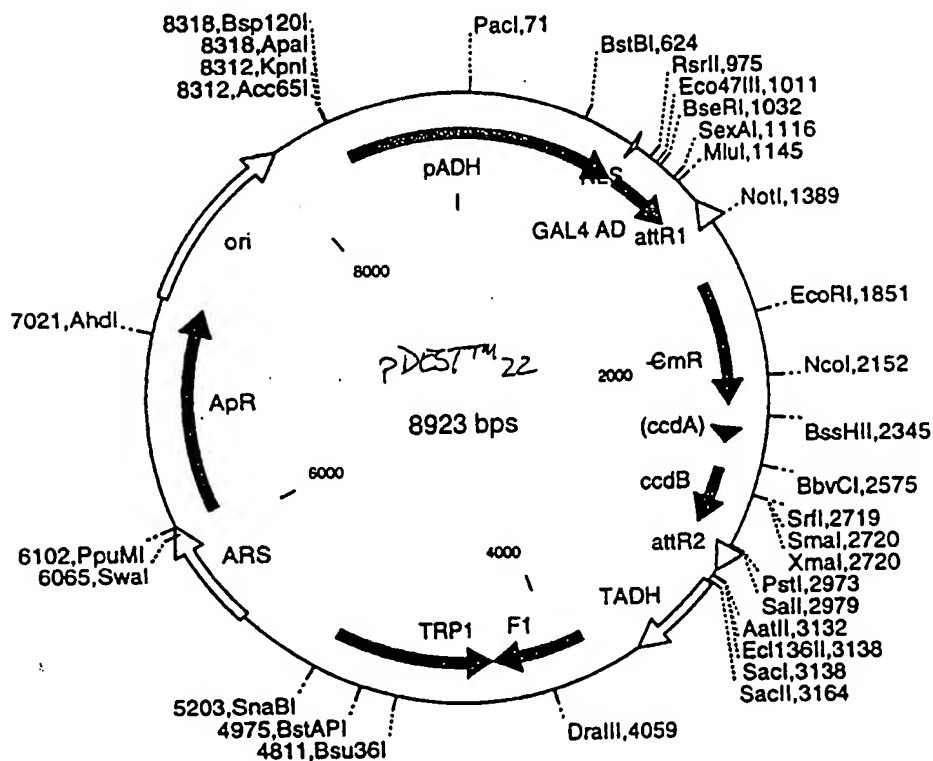
810 ~~ccc/ttg ttt ctt ttt ctg cat gat att tca agc tat acc aag cat acc atc~~  
~~agg aac aaa gaa aaa gac gtg tta taa agt tgc ata tgg ttc gta tat tag~~

861 ~~aac/ttc aag ctt atg ccc aag aag cgg aag gtc tgc agc ggc gcc aat~~  
~~ttg agg ttc gaa tac ggg ttc ttc ttc gcc ttc cag agc tgc ccg cgg tta~~

1218 gaa gat acc cca cca aac cca aaa aaa <sup>D G G S N Q T S</sup> gag ggt ggg tgg aat caa <sup>aca agt</sup>  
ctt cta tgg ggt ggt ttg ggt ttt ttt ctc cca ccc agc tta gtt <sup>tgt tca</sup>

1269 ~~ttg tac aaa aaa gct gaa cga gaa acg taa a~~  
~~aac atg ttt ttt cga ctt gct ctt tgc att t~~

ADH Promoter  
Gal4-AD  
Start Translation  
Int



117/240

## pDEST22 8923 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
904..1248		GAL4 AD
1388..1264		attR1
1638..2297		CmR
2417..2501		inactivated ccdA
2639..2944		ccdB
2985..3109		attR2
3831..4318		f1 (f1 intergenic region)
4334..5176		TRP1
6110..7194		ampR
8344..866		pADH (yeast ADH promoter)

1	TTCATTTGGG	TGTGCACTTT	ATTATGTTAC	AATATGGAAG	GGAACCTTTAC	ACTTCTCCTA
61	TGCACATATA	TTAATTAAAG	TCCAATGCTA	GTAGAGAAGG	GGGGTAACAC	CCCTCCGCGC
121	TCTTTTCCGA	TTTTTTTCTA	AACCGTGGAA	TATTTTCGGAT	ATCCTTTTGT	TGTTTCCGGG
181	TGTACAATAT	GGACTTCCTC	TTTTCTGGCA	ACCAAACCCA	TACATCGGGA	TTCCTATAAT
241	ACCTTCGTTG	GTCTCCCTAA	CATGTAGGTG	GCGGAGGGGA	GATATACAAT	AGAACAGATA
301	CCAGACAAGA	CATAATGGGC	TAAACAAGAC	TACACCAATT	AACTGCCTC	ATTGATGGTG
361	GTACATAACG	AACTAATACT	GTAGCCCTAG	ACTTGATAGC	CATCATCATA	TCGAAGTTTC
421	ACTACCCTTT	TTCCATTTCG	CATCTATTGA	AGTAATAATA	GGCGCATGCA	ACTTCTTTTC
481	TTTTTTTTTC	TTTTCTCTCT	CCCCGTTTGT	TGTCTCACCA	TATCCGCAAT	GACAAAAAAA
541	ATGATGGAAG	ACACTAAAGG	AAAAAATTAA	CGACAAAGAC	AGCACCAACA	GATGTCGTTG
601	TTCCAGAGCT	GATGAGGGGT	ATCTTCGAAC	ACACGAAACT	TTTTCTTTCC	TTCATTACAG
661	CACACTACTC	TCTAATGAGC	AACGGTATAC	GGCCTTCCTT	CCAGTTACTT	GAATTTGAAA
721	TAAAAAAGT	TTGCCGCTTT	GCTATCAAGT	ATAAATAGAC	CTGCAATTAT	TAATCTTTTG
781	TTTCTCTGTC	ATTGTTCTCG	TTCCCTTTCT	TCCTTGTTTC	TTTTCTTGCA	CAATATTTC
841	AGCTATACCA	AGCATACAAT	CAACTCCAAG	CTTATGCCCA	AGAAGAAGCG	GAAGGTCTCG
901	AGCGGCGCCA	ATTTAATCA	AAGTGGGAAT	ATTGCTGATA	GCTCATTGTC	CTTCACTTTC
961	ACTAACAGTA	GCAACGGTCC	GAACCTCATA	ACAACCTCAA	CAAATTCTCA	AGCGCTTTCA
1021	CAACCAATTG	CCTCCTCTAA	CGTTCATGAT	AACTTCATGA	ATAATGAAAT	CACGGCTAGT
1081	AAAATTGATG	ATGGTAATAA	TTCAAAACCA	CTGTACCTTG	GTTGGACGGA	CCAAACTGCG
1141	TATAACGCGT	TTGGAATCAC	TACAGGGATG	TTTAATACCA	CTACAATGGA	TGATGTATAT
1201	AACTATCTAT	TCGATGATGA	AGATACCCCA	CCAAACCCAA	AAAAAGAGGG	TGGGTCGAAT
1261	CAAACAAGTT	TGTACAAAAA	AGCTGAACGA	GAAACGTAAA	ATGATATAAA	TATCAATATA
1321	TTAAATTAGA	TTTTGCATAA	AAAACAGACT	ACATAATACT	GTAACACACA	ACATATCCAG
1381	TCACATATGC	GGCCGCTAAG	TGGCAGCAT	CACCCGACGC	ACTTTGCGCC	GAATAAATAC
1441	CTGTGACGGA	AGATCACTTC	GCAGAATAAA	TAAATCCTGG	TGTCCCTGTT	GATACCGGGA
1501	AGCCCTGGGC	CAACTTTTGG	CGAAAATGAG	ACGTTGATCG	GCACGTAAGA	GGTTCCAACT
1561	TTCACCATAA	TGAAATAAGA	TCACTACCGG	GCGTATTTTT	TGAGTTATCG	AGATTTTCAG
1621	GAGCTAAGGA	AGCTAAAATG	GAGAAAAAAA	TCACTGGATA	TACCACCGTT	GATATATCCC
1681	AATGGCATCG	TAAAGAACAT	TTTGAGGCAT	TTCACTCAGT	TGCTCAATGT	ACCTATAACC
1741	AGACCGTTCA	GCTGGATATT	ACGGCCTTTT	TAAAGACCGT	AAAGAAAAAT	AAGCACAAAGT
1801	TTTATCCGGC	CTTTATTTCAC	ATTCTTGCCC	GCCTGATGAA	TGCTCATCCG	GAATTCCGTA
1861	TGGCAATGAA	AGACGGTGAG	CTGGTGATAT	GGGATAGTGT	TCACCCTTGT	TACACCGTTT
1921	TCCATGAGCA	AACTGAAACG	TTTTCATCGC	TCTGGAGTGA	ATACCACGAC	GATTTCCGGC
1981	AGTTTCTACA	CATATATTCC	CAAGATGTGG	CGTGTACGG	TGAAAACCTG	GCCTATTTC
2041	CTAAAGGGTT	TATTGAGAAT	ATGTTTTTCG	TCTCAGCCAA	TCCCTGGGTG	AGTTTCACCA
2101	GTTTTGATTT	AAACGTGGCC	AATATGGACA	ACTTCTTCGC	CCCCGTTTTT	ACCATGGGCA
2161	AATATTATAC	GCAAGGCGAC	AAGGTGCTGA	TGCCGCTGGC	GATTACAGTT	CATCATGCCG
2221	TCTGTGATGG	CTTCCATGTC	GGCAGAATGC	TTAATGAATT	ACAACAGTAC	TGCGATGAGT
2281	GGCAGGGCGG	GGCGTAATCT	AGAGGATCCG	GCTTACTAAA	AGCCAGATAA	CAGTATGCGT
2341	ATTTGCGCGC	TGATTTTTCG	GGTATAAGAA	TATATACTGA	TATGTATACC	CGAAGTATGT
2401	GAAAAAGAGG	TGTGCTATGA	AGCAGCGTAT	TACAGTGACA	GTTGACAGCG	ACAGCTATCA
2461	CTTGCTCAAG	GCATATATGA	TGTCAATATC	TCCGGTCTGG	TAAGCACAAC	CATGCAGAAT
2521	GAAGCCCGTC	GTCTGCGTGC	CGAACGCTGG	AAAGCGGAAA	ATCAGGAAGG	GATGGCTGAG-

FIGURE 42B

2581 GTCGCCCGGT TTATTGAAAT GAACGGCTCT TTTGCTGACG AGAACAGGGA CTGGTGAAAT  
2641 GCAGTTTAAG GTTTACACCT ATAAAAGAGA GAGCCGTTAT CGTCTGTTTG TGGATGTACA  
2701 GAGTGATATT ATTGACACGC CCGGGCGACG GATGGTGATC CCCCTGGCCA GTGCACGTCT  
2761 GCTGTCAGAT AAAGTCTCCC GTGAACTTTA CCCGGTGGTG CATATCGGGG ATGAAAGCTG  
2821 GCGCATGATG ACCACCGATA TGGCCAGTGT GCCGGTCTCC GTTATCGGGG AAGAAGTGGC  
2881 TGATCTCAGC CACCGCGAAA ATGACATCAA AAACGCCATT AACCTGATGT TCTGGGGAAT  
2941 ATAAATGTCA GGCTCCCTTA TACACAGCCA GTCTGCAGGT CGACCATAGT GACTGGATAT  
3001 GTTGTGTTTT ACAGTATTAT GTAGTCTGTT TTTTATGCAA AATCTAATTT AATATATTGA  
3061 TATTTATATC ATTTTACGTT TCTCGTTCAG CTTTCTTGTA CAAAGTGGTT TGATGGCCGC  
3121 TAAGTAAGTA AGACGTCGAG CTCTAAGTAA GTAACGGCCG CCACCGCGGT GGAGCTTTGG  
3181 ACTTCTTCGC CAGAGGTTTG GTCAAGTCTC CAATCAAGGT TGTCGGCTTG TCTACCTTGC  
3241 CAGAAATTTA CGAAAAGATG GAAAAGGGTC AAATCGTTGG TAGATACGTT GTTGACACTT  
3301 CTAAATAAGC GAATTTCTTA TGATTTATGA TTTTATTAT TAAATAAGTT ATAAAAAAA  
3361 TAAGTGATA CAAATTTTAA AGTGACTCTT AGGTTTAAA ACGAAAATTC TTATTCTTGA  
3421 GTAACCTCTT CCTGTAGGTC AGGTTGCTTT CTCAGGTATA GCATGAGGTC GCTCTTATTG  
3481 ACCACACCTC TACCGGCATG CCGAGCAAAT GCCTGCAAAT CGCTCCCCAT TTCACCCAAT  
3541 TGTAGATATG CTAAGTCCAG CAATGAGTTG ATGAATCTCG GTGTGTATTT TATGTCTTCA  
3601 GAGGACAATA CCTGTTGTAA TCGTCTTTC ACACGGATCC CAATTCGCCC TATAGTGAGT  
3661 CGTATTACAA TTCACTGGCC GTCGTTTTAC AACGTCGTGA CTGGGAAAAC CCTGGCGTTA  
3721 CCCAACTTAA TCGCCTTGCA GCACATCCCC CTTTCGCCAG CTGGCGTAAT AGCGAAGAGG  
3781 CCCGCACCGA TCGCCCTTCC CAACAGTTGC GCAGCCTGAA TGGCGAATGG ACGCGCCCTG  
3841 TAGCGGCGCA TTAAGCGCGG CGGGTGTGGT GGTTACGCGC AGCGTGACCG CTACACTTGC  
3901 CAGCGCCCTA GCGCCCGCTC CTTTCGCTTT CTTCCCTTCC TTTCTCGCCA CGTTCGCCGG  
3961 CTTTCCCGCT CAAGCTCTAA ATCGGGGGCT CCCTTTAGGG TTCCGATTTA GTGCTTTACG  
4021 GCACCTCGAC CCCAAAAAAC TTGATTAGGG TGATGGTTCA CGTAGTGGGC CATCGCCCTG  
4081 ATAGACGGTT TTTCCGCCCT TGACGTTGGA GTCCACGTTT TTTAATAGTG GACTCTTGTT  
4141 CCAAAGTGA ACAACACTCA ACCCTATCTC GGTCTATTCT TTTGATTTAT AAGGGATTTT  
4201 GCCGATTTTC GCCTATTGGT TAAAAAATGA GCTGATTAA CAAAAATTTA ACGCGAATTT  
4261 TAACAAAATA TTAACGTTTA CAATTTCCCTG ATGCGGTATT TTCTCCTTAC GCATCTGTGC  
4321 GGTATTTTAC ACCGCAGGCA AGTGACAAA CAATACTTAA ATAAATACTA CTCAGTAATA  
4381 ACCTATTTCT TAGCATTTTT GACGAAATTT GCTATTTTGT TAGAGTCTTT TACACCATTT  
4441 GTCTCCACAC CTCCCGTTAC ATCAACACCA ATAACGCCAT TTAATCTAAG CGCATACCA  
4501 ACATTTTCTG GCGTCAGTCC ACCAGCTAAC ATAAATGTA AGCTTTCGGG GCTCTCTTGC  
4561 CTTCCAACCC AGTCAGAAAT CGAGTTCCAA TCCAAAAGTT CACCTGTCCC ACCTGCTTCT  
4621 GAATCAACA AGGGAATAAA CGAATGAGGT TTCTGTGAAG CTGCACTGAG TAGTATGTTG  
4681 CAGTCTTTTG GAAATACGAG TCTTTTAATA ACTGGCAAAC CGAGGAACTC TTGGTATTCT  
4741 TGCCACGACT CATCTCCATG CAGTTGGACG ATATCAATGC CGTAATCATT GACCAGGCC  
4801 AAAACATCCT CCTTAGGTTG ATTACGAAAC ACGCCAACCA AGTATTTCCG AGTGCTGAA  
4861 CTATTTTAT ATGCTTTTAC AAGACTTGAA ATTTTCCTTG CAATAACCGG GTCAATTGTT  
4921 CTCTTTCTAT TGGGCACACA TATAATACCC AGCAAGTCAG CATCGGAATC TAGAGCACAT  
4981 TCTGCGGCCT CTGTGCTCTG CAAGCCGCAA ACTTTCACCA ATGGACCAGA ACTACCTGTG  
5041 AAATTAATAA CAGACATACT CCAAGCTGCC TTTGTGTGCT TAATCACGTA TACTCACGTG  
5101 CTCAATAGTC ACCAATGCCC TCCCTCTTGG CCCTCTCCTT TTCTTTTTTC GACCGAATTA  
5161 ATTCTTAATC GGCAAAAAA GAAAAGCTCC GGATCAAGAT TGTACGTAAG GTGACAAGCT  
5221 ATTTTCAAT AAAGAATATC TTCCACTACT GCCATCTGGC GTCATACTG CAAAGTACAC  
5281 ATATATTACG ATGCTGTCTA TTAAATGCTT CCTATATTAT ATATATAGTA ATGTCGTTTA  
5341 TGGTGCACTC TCAGTACAAT CTGCTCTGAT GCCGCATAGT TAAGCCAGCC CCGACACCCG  
5401 CCAACACCCG CTGACGCGCC CTGACGGGCT TGTCTGCTCC CGGCATCCGC TTACAGACAA  
5461 GCTGTGACCG TCTCCGGGAG CTGCATGTGT CAGAGGTTT CACCGTCATC ACCGAAACGC  
5521 GCGAGACGAA AGGGCCTCGT GATACGCCTA TTTTATAGG TTAATGTCAT GATAATAATG  
5581 GTTCTTAGG ACGGATCGCT TGCTGTAAAC TTACACGCGC CTCGTATCTT TTAATGATGG  
5641 AATAATTTGG GAATTTACTC TGTGTTTATT TATTTTATG TTTTGTATT GGATTTTGA  
5701 AAGTAAATAA AGAAGGTAGA AGAGTTACGG AATGAAGAAA AAAAAATAAA CAAAGGTTTA  
5761 AAAAAATTTCA AAAAAAGCG TACTTTACAT ATATATTTAT TAGACAAGAA AAGCAGATTA  
5821 AATAGATATA CATTGATTA ACGATAAGTA AAATGTAAAA TCACAGGATT TTCGTGTGTG  
5881 GTCTTCTACA CAGACAAGAT GAAACAATTC GGCATTAATA CCTGAGAGCA GGAAGAGCAA  
5941 GATAAAAGGT AGTATTTGTT GGCGATCCCC CTAGAGTCTT TTACATCTTC GGAAAAA  
6001 AACTATTTTT TCTTTAATTT CTTTTTTTAC TTTCTATTTT TAATTTATAT ATTTATATTA-

119/240

6061 AAAAATTAA ATTATAATTA TTTTATAGC ACGTGATGAA AAGGACCCAG GTGGCACTTT  
6121 TCGGGGAAAT GTGCGCGGAA CCCCTATTG TTTATTTTTC TAAATACATT CAAATATGTA  
6181 TCCGCTCATG AGACAATAAC CCTGATAAAT GCTTCAATAA TATTGAAAAA GGAAGAGTAT  
6241 GAGTATTCAA CATTTCCTG TCGCCCTTAT TCCCTTTTTT GCGGCATTTT GCCTTCCTGT  
6301 TTTTGCTCAC CCAGAAACGC TGGTGAAAGT AAAAGATGCT GAAGATCAGT TGGGTGCACG  
6361 AGTGGGTAC ATCGAACTGG ATCTCAACAG CCGTAAGATC CTTGAGAGTT TTCGCCCCGA  
6421 AGAACGTTTT CCAATGATGA GCACTTTTAA AGTTCTGCTA TGTGGCGCGG TATTATCCCG  
6481 TATTGACGCC GGGCAAGAGC AACTCGGTGC CCGCATACAC TATTCTCAGA ATGACTTGGT  
6541 TGAGTACTCA CCAGTCACAG AAAAGCATCT TACGGATGGC ATGACAGTAA GAGAATTATG  
6601 CAGTGCTGCC ATAACCATGA GTGATAACAC TGCGGCCAAC TTACTTCTGA CAACGATCGG  
6661 AGGACCGAAG GAGCTAACCG CTTTTTTTCA CAACATGGGG GATCATGTAA CTCGCCCTGA  
6721 TCGTTGGGAA CCGGAGCTGA ATGAAGCCAT ACCAAACGAC GAGCGTGACA CCACGATGCC  
6781 TGTAGCAATG GCAACAACGT TCGCAAACCT ATTAACCTGC GAACACTTAA CTCTAGCTTC  
6841 CCGGCAACAA TTAATAGACT GGATGGAGGC GGATAAAGTT GCAGGACCAC TTCTGCGCTC  
6901 GGCCCTTCCG GCTGGCTGGT TTATTGCTGA TAAATCTGGA GCCGGTGAGC GTGGGTCTCG  
6961 CGGTATCATT GCAGCACTGG GGCCAGATGG TAAGCCCTCC CGTATCGTAG TTATCTACAC  
7021 GACGGGCAGT CAGGCAACTA TGGATGAACG AAATAGACAG ATCGCTGAGA TAGGTGCCTC  
7081 ACTGATTAAG CATTGGTAAC TGTCAGACCA AGTTTACTCA TATATACTTT AGATTGATTT  
7141 AAAACTTCAT TTTTAATTTA AAAGGATCTA GGTGAAGATC CTTTTTGATA ATCTCATGAC  
7201 CAAAATCCCT TAACGTGAGT TTTCTGTTCCA CTGAGCGTCA GACCCCGTAG AAAAGATCAA  
7261 AGGATCTTCT TGAGATCCTT TTTTCTGCG CGTAATCTGC TGCTTGCAAA CAAAAAACC  
7321 ACCGCTACCA GCGGTGGTTT GTTTGCCGGA TCAAGAGCTA CCAACTCTTT TTCCGAAGGT  
7381 AACTGGCTTC AGCAGAGCGC AGATACCAAA TACTGTCTTT CTAGTGTAGC CGTAGTTAGG  
7441 CCACCACTTC AAGAACTCTG TAGCACCGCC TACATACCTC GCTCTGCTAA TCCTGTTACC  
7501 AGTGGCTGCT GCCAGTGCGG ATAAGTCGTG TCTTACCGGG TTGGACTCAA GACGATAGTT  
7561 ACCGATAAG GCGCAGCGGT CCGGCTGAAC GGGGGGTTTCG TGCACACAGC CCAGCTTGGA  
7621 GCGAACGACC TACACCGAAC TGAGATACCT ACAGCGTGAG CATTGAGAAA GCGCCACGCT  
7681 TCCCGAAGGG AGAAAGGCGG ACAGGTATCC GGTAAGCGGC AGGGTCGGAA CAGGAGAGCG  
7741 CACGAGGGAG CTTCCAGGGG GGAACGCCTG GTATCTTTAT AGTCCTGTGC GGTTCGCCA  
7801 CCTCTGACTT GAGCGTCGAT TTTTGTGATG CTCGTCAGGG GGGCCGAGCC TATGAAAAA  
7861 CGCCAGCAAC GCGGCCTTTT TACGGTTCCT GGCTTTTTCG TGGCCTTTG CTCACATGTT  
7921 CTTTCTGCG TTATCCCCTG ATTCTGTGGA TAACCGTATT ACCGCCTTTG AGTGAGCTGA  
7981 TACCGCTCGC CGCAGCCGAA CGACCGAGCG CAGCGAGTCA GTGAGCGAGG AAGCGGAAGA  
8041 GCGCCCAATA CGCAAACCGC CTCTCCCCGC GCGTTGGCCG ATTCATTAAAT GCAGCTGGCA  
8101 CGACAGGTTT CCCGACTGGA AAGCGGGCAG TGAGCGCAAC GCAATTAATG TGAGTTACCT  
8161 CACTCATTAG GCACCCAGG CTTTACACTT TATGCTTCCG GCTCCTATGT TGTGTGGAAT  
8221 TGTGAGCGGA TAACAATTTT ACACAGGAAA CAGCTATGAC CATGATTACG CCAAGCTCGG  
8281 AATTAACCTT CACTAAAGGG AACAAAAGCT GGGTACCGGG CCCCCCTCG AGATCCGGGA  
8341 TCGAAGAAAT GATGGTAAAT GAAATAGGAA ATCAAGGAGC ATGAAGGCAA AAGACAAATA  
8401 TAAGGGTCGA ACGAAAAATA AAGTGAAGAG TGTTGATATG ATGTATTTGG CTTTGCGGCG  
8461 CCGAAAAAAC GAGTTTACGC AATTGCACAA TCATGCTGAC TCTGTGGCGG ACCCGCGCTC  
8521 TTGCCGGCCC GCGGATAACG CTGGGCGTGA GGCTGTGCCC GCGGAGTTT TTTGCGCCTG  
8581 CATTTTCCAA GGTTTACCCT GCGCTAAGGG GCGAGATTGG AGAAGCAATA AGAATGCCGG  
8641 TTGGGGTTGC GATGATGACG ACCACGACAA CTGGTGTCAT TATTAAAGTT GCCGAAAGAA  
8701 CCTGAGTGCA TTTGCAACAT GAGTATACTA GAAGAATGAG CCAAGACTTG CGAGACGCGA  
8761 GTTTGCCGGT GGTGCGAACA ATAGAGCGAC CATGACCTTG AAGGTGAGAC GCGCATAACC  
8821 GCTAGAGTAC TTTGAAGAGG AAACAGCAAT AGGGTTGCTA CCAGTATAAA TAGACAGGTA  
8881 CATAACAAC TGGAAATGGT TGTCTGTTTG AGTACGCTTT CAA

Figure 420

120/240

pDEST23

His6 carboxy-fusion vector, T7 promoter

205    atc ccg cga aat taa tac gac tca cta tag gga gat cac aac ggt ttc cct MRNA  
      tag ggc gct tta att atg ctg agt gat atc cgt ctg gtg ttg cca aag gga  
 256    cta gat caa aag ttt gta caa aaa agc tga acg aga aac gta aaa tga tat att R1  
      gat cta gtg ttc aaa cat gtt ttt tgg act tgc tct ttg cat ttt act ata

1888    ttt tta tgc aaa atc taa ttt aat ata ttg ata ttt ata tca ttt tao gtt  
      aaa aat acg ttt tag att aaa tta tat aac tat aaa tat agt aaa atg caa  
 1939    att R2 A F L Y K V V I M S Y Y H H  
      tct cgt tca gct ttc ttg tac aaa gtg gtg att atg tgc tac tac cat cac  
      aga gca agt cga aag aac atg ttt cac cac taa tac agc atg atg gta gtg  
 1990    H H H H L D E V Q term HIS6  
      cat cat cat cac ctg gat gag caa taa cta gca taa ccc ctt ggg gcc tct  
      gta gtg gta gtg gag cta ctc gtt att gat cgt att ggg gaa ccc cgg aga

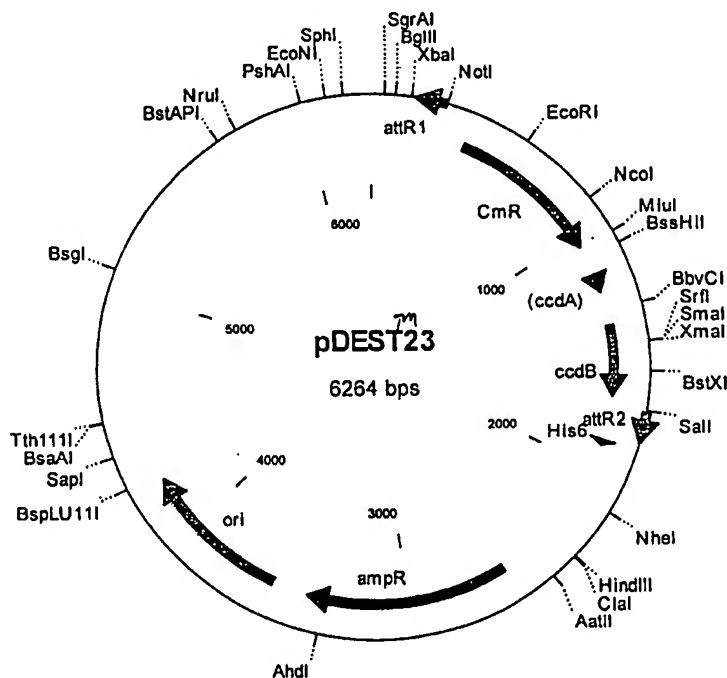


FIGURE 43A

121/240

## pDEST23 6264 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
285..161		attR1
394..1053		CmR
1173..1257		inactivated ccdA
1395..1700		ccdB
1741..1865		attR2
1883..1911		his6
2574..3434		ampR
3583..4222		ori

1	TCTTCCCAT	CGGTGATGTC	GGCGATATAG	GCGCCAGCAA	CCGCACCTGT	GGCGCCGGTG
61	ATGCCGGCCA	CGATGCGTCC	GGCGTAGAGG	ATCGAGATCT	CGATCCC CGG	AAATTAATAC
121	GACTCACTAT	AGGGAGACCA	CAACGGTTTC	CCTCTAGATC	ACAAGTTTGT	ACAAAAAAGC
181	TGAACGAGAA	ACGTAAAATG	ATATAAATAT	CAATATATTA	AATTAGATTT	TGCATAAAAA
241	ACAGACTACA	TAATACTGTA	AAACACAACA	TATCCAGTCA	CTATGGCGGC	CGCATTAGGC
301	ACCCAGGCT	TTACACTTTA	TGCTTCCGGC	TCGTATAATG	TGTGGATTTT	GAGTTAGGAT
361	CCGCGGAGAT	TTTCAGGAGC	TAAGGAAGCT	AAAATGGAGA	AAAAAATCAC	TGGATATACC
421	ACCGTTGATA	TATCCCAATG	GCATCGTAAA	GAACATTTTG	AGGCATTTCA	GTCAGTTGCT
481	CAATGTACCT	ATAACCAGAC	CGTTCAGCTG	GATATTACGG	CCTTTTAA	GACCGTAAAG
541	AAAAATAAGC	ACAAGTTTAA	TCCGGCCTTT	ATTCACATTC	TTGCCCGCCT	GATGAATGCT
601	CATCCGGAAT	TCCGTATGGC	AATGAAAGAC	GGTGAGCTGG	TGATATGGGA	TAGTGTTCAC
661	CCTTGTTACA	CCGTTTTCCA	TGAGCAAAC	GAAACGTTTT	CATCGCTCTG	GAGTGAATAC
721	CACGACGATT	TCCGGCAGTT	TCTACACATA	TATTCGCAAG	ATGTGGCGTG	TTACGGTGAA
781	AACCTGGCCT	ATTTCCCTAA	AGGGTTTATT	GAGAATATGT	TTTTCGTCTC	AGCCAATCCC
841	TGGGTGAGTT	TCACCAGTTT	TGATTTAAAC	GTGGCCAATA	TGGACAACTT	CTTCGCCCCC
901	GTTTTCACCA	TGGGCAAATA	TTATACGCAA	GGCGACAAGG	TGCTGATGCC	GCTGGCGATT
961	CAGGTTTCATC	ATGCCGTCTG	TGATGGCTTC	CATGTCGGCA	GAATGCTTAA	TGAATTACAA
1021	CAGTACTGCG	ATGAGTGCGA	GGGCGGGGCG	TAAACGCGTG	GATCCGGCTT	ACTAAAAGCC
1081	AGATAACAGT	ATGCGTATTT	GCGCGCTGAT	TTTTGCGGTA	TAAGAATATA	TACTGATATG
1141	TATACCCGAA	GTATGTCAAA	AAGAGGTGTG	CTATGAAGCA	GCGTATTACA	GTGACAGTTG
1201	ACAGCGACAG	CTATCAGTTG	CTCAAGGCAT	ATATGATGTC	AATATCTCCG	GTCTGGTAAG
1261	CACAACCATG	CAGAATGAAG	CCCGTCGTCT	GCGTGCCGAA	CGCTGGAAAG	CGGAAAATCA
1321	GGAAGGGATG	GCTGAGGTCG	CCCGGTTTAT	TGAAATGAAC	GGCTCTTTTG	CTGACGAGAA
1381	CAGGGACTGG	TGAAATGCAG	TTTAAGGTTT	ACACCTATAA	AAGAGAGAGC	GCTTATCGTC
1441	TGTTTGTTGA	TGTACAGAGT	GATATTATTG	ACACGCCCCG	GCGACGGATG	GTGATCCCCC
1501	TGGCCAGTGC	ACGTCTGCTG	TCAGATAAAG	TCTCCCGTGA	ACTTTACCCG	GTGGTGCATA
1561	TCGGGGATGA	AAGCTGGCGC	ATGATGACCA	CCGATATGGC	CAGTGTGCCG	GTCTCCGTTA
1621	TCGGGGAAGA	AGTGGCTGAT	CTCAGCCACC	GCGAAAATGA	CATCAAAAAC	GCCATTAAAC
1681	TGATGTTCTG	GGGAATATAA	ATGTCAGGCT	CCCTTATACA	CAGCCAGTCT	GCAGGTGCGAC
1741	CATAGTGACT	GGATATGTTG	TGTTTTACAG	TATTATGTAG	TCTGTTTTTT	ATGCAAAATC
1801	TAATTTAATA	TATTGATATT	TATATCATTT	TACGTTTCTC	GTTCAGCTTT	CTTGTAACAA
1861	GTGGTGATTA	TGTCGTACTA	CCATCACCAT	CACCATCACC	TCGATGAGCA	ATAAGTAGCA
1921	TAACCCCTTG	GGGCCTCTAA	ACGGGTCTTG	AGGGGTTTTT	TGCTGAAAGG	AGGAACATA
1981	TCCGGATATC	CACAGGACGG	GTGTGGTCGC	CATGATCGCG	TAGTCGATAG	TGGCTCCAAG
2041	TAGCGAAGCG	AGCAGGACTG	GGCGGCGGCC	AAAGCGGTCTG	GACAGTGCTC	CGAGAACGGG
2101	TGCCCATAGA	AATTGCATCA	ACGCATATAG	CGCTAGCAGC	ACGCCATAGT	GACTGGCGAT
2161	GCTGTCCGAA	TGGACGATAT	CCCGCAAGAG	GCCCCGCGAGT	ACCGGCATAA	CCAAGCCTAT
2221	GCCTACAGCA	TCCAGGGTGA	CGGTGCCGAG	GATGACGATG	AGCGCATTGT	TAGATTTCAT
2281	ACACGGTGCC	TGACTGCGTT	AGCAATTTAA	CTGTGATAAA	CTACCGCATT	AAAGCTTATC
2341	GATGATAAGC	TGTCAAACAT	GAGAATTCTT	GAAGAAGAAA	GGGCCTCGTG	ATACGCCCTAT
2401	TTTTATAGGT	TAATGTCATG	ATAATAATGG	TTTCTTAGAC	GTCAGGTGGC	ACTTTTCGGG
2461	GAAATGTGCG	CGGAACCCCT	ATTTGTTTAT	TTTTCTAAAT	ACATTCAAAT	ATGTATCCGC
2521	TCATGAGACA	ATAACCCCTGA	TAAATGCTTC	AATAATATTG	AAAAAGGAAG	AGTATGAGTA
2581	TTCAACATTT	CCGTGTCGCC	CTTATTCCCT	TTTTTGCGGC	ATTTTGCCCTT	CCTGTTTTTG
2641	CTCACCCAGA	AACGCTGGTG	AAAGTAAAAG	ATGCTGAAGA	TCAGTTGGGT	GCACGAGTGG

FIGURE 43B

122/240

```

2701 GTTACATCGA ACTGGATCTC AACAGCGGTA AGATCCTTGA GAGTTTTTCGC CCCGAAGAAC
2761 GTTTTCCAAT GATGAGCACT TTTAAAGTTC TGCTATGTGG CGCGGTATTA TCCCGTGTG
2821 ACGCCGGGCA AGAGCAACTC GGTCCGCCGA TACACTATTC TCAGAATGAC TTGGTTGAGT
2881 ACTCACCAGT CACAGAAAAG CATCTTACGG ATGGCATGAC AGTAAGAGAA TTATGCAGTG
2941 CTGCCATAAC CATGAGTGAT AACACTGCGG CCAACTTACT TCTGACAACG ATCGGAGGAC
3001 CGAAGGAGCT AACCGCTTTT TTGCACAACA TGGGGGATCA TGTAACTCGC CTGTATCGTT
3061 GGGAAACCGGA GCTGAATGAA GCCATACCAA ACGACGAGCG TGACACCACG ATGCCTGCAG
3121 CAATGGCAAC AACGTTGCGC AAACATTTAA CTGGCGAACT ACTTACTCTA GCTTCCCGGC
3181 AACAAATTAAT AGACTGGATG GAGGCGGATA AAGTTGCAGG ACCACTTCTG CGCTCGGCCC
3241 TTCCGGCTGG CTGGTTTATT GCTGATAAAT CTGGAGCCGG TGAGCGTGGG TCTCGCGGTA
3301 TCATTGCAGC ACTGGGGCCA GATGGTAAGC CCTCCCGTAT CGTAGTTATC TACACGACGG
3361 GGAGTCAGGC AACTATGGAT GAACGAAATA GACAGATCGC TGAGATAGGT GCCTCACTGA
3421 TTAAGCATTG GTAACGTGCA GACCAAGTTT ACTCATATAT ACTTTAGATT GATTTAAAC
3481 TTCATTTTAA ATTTAAAGG ATCTAGGTGA AGATCCTTTT TGATAATCTC ATGACCAAAA
3541 TCCCTTAACG TGAGTTTTCG TTCCACTGAG CGTCAGACCC CGTAGAAAAG ATCAAAGGAT
3601 CTTCTTGAGA TCCTTTTTTT CTGCGCGTAA TCTGCTGCTT GCAAACAAAA AAACCACCGC
3661 TACCAGCGGT GGTGTGTTTG CCGGATCAAG AGCTACCAAC TCTTTTTCCG AAGGTAACGT
3721 GCTTCAGCAG AGCGCAGATA CCAAATACTG TCCTTCTAGT GTAGCCGTAG TTAGGCCACC
3781 ACTTCAAGAA CTCTGTAGCA CCGCTACAT ACCTCGCTCT GCTAATCCTG TTACCAGTGG
3841 CTGCTGCCAG TGGCGATAAG TCGTGTCTTA CCGGGTTGGA CTCAAGACGA TAGTTACCGG
3901 ATAAGCGCAG GCGGTCGGGC TGAACGGGGG GTTCGTGCAC ACAGCCCAGC TTGGAGCGAA
3961 CGACCTACAC CGAAGTGAGA TACCTACAGC GTGAGCTATG AGAAAGCGCC ACGCTTCCCG
4021 AAGGGAGAAA GGCGGACAGG TATCCGGTAA GCGGCAGGGT CGGAACAGGA GAGCGCACGA
4081 GGGAGCTTCC AGGGGGAAC GCCTGGTATC TTTATAGTCC TGTCGGGTTT CGCCACCTCT
4141 GACTTGAGCG TCGATTTTTG TGATGCTCGT CAGGGGGGCG GAGCCTATGG AAAACGCCA
4201 GCAACGCGGC CTTTTTACGG TTCCTGGCCT TTTGCTGGCC TTTTGCTCAC ATGTTCTTTC
4261 CTGCGTTATC CCCTGATTCT GTGGATAACC GTATTACCGC CTTTGAGTGA GCTGATACCG
4321 CTCGCGCAG CGGAACGACC GAGCGCAGCG AGTCAGTGAG CGAGGAAGCG GAAGAGCGCC
4381 TGATGCGGTA TTTTCTCCTT ACGCATCTGT GCGGTATTTT ACACCGCATA TATGGTGCAC
4441 TCTCAGTACA ATCTGCTCTG ATGCCGCATA GTTAAGCCAG TATACTCTCC GCTATCGCTA
4501 CGTGAAGTGG TCATGGCTGC GCGCCGACAC CCGCCAACAC CCGCTGACGC GCCCTGACGG
4561 GCTTGCTGCG TCCCGGCATC CGCTTACAGA CAAGCTGTGA CCGTCTCCGG GAGCTGCATG
4621 TGTGAGAGGT TTTCACCGTC ATCACCAGAA CGCGCGAGGC AGCTGCGGTA AAGCTCATCA
4681 GCGTGGTCTG GAAGCGATTG ACAGATGTCT GCCTGTTTCAT CCGCGTCCAG CTCGTTGAGT
4741 TTCTCCAGAA GCGTTAATGT CTGGCTTCTG ATAAAGCGGG CCATGTTAAG GCGGTTTTC
4801 TCCTGTTTGG TCACTGATGC CTCGCTGTAA GGGGGATTTT TGTTTCATGG GGTAAATGATA
4861 CCGATGAAAC GAGAGAGGAT GCTCACGATA CCGGTTACTG ATGATGAACA TGCCCGGTTA
4921 CTGGAACGTT GTGAGGGTAA ACAACTGGCG GTATGGATGC GCGGGGACCA GAGAAAAATC
4981 ACTCAGGGTC AATGCCAGCG CTTCTGTTAAT ACAGATGTAG GTGTTCCACA GGGTAGCCAG
5041 CAGCATCCTG CGATGCAGAT CCGGAACATA ATGGTGAGG GCGCTGACTT CCGCGTTTCC
5101 AGACTTTACG AAACACGGAA ACCGAAGACC ATTCATGTTG TTGCTCAGGT CGCAGACGTT
5161 TTGCAGCAGC AGTCGCTTCA CGTTCGCTCG CGTATCGGTG ATTCATTCTG CTAACCAGTA
5221 AGGCAACCCC GCCAGCCTAG CCGGTCCTC AACGACAGGA GCACGATCAT GCGCACCCGT
5281 GGCCAGGACC CAACGCTGCC CGAGATGCGC CGCGTGCGGC TGCTGGAGAT GCGGACCGC
5341 ATGGATATGT TCTGCCAAGG GTTGGTTTGC GCATTACAG TTCTCCGCAA GAATTGATTG
5401 GCTCCAATTC TTGGAGTGGT GAATCCGTTA GCGAGGTGCC GCCGGCTTCC ATTCAGGTCC
5461 AGGTGGCCCC GCTCCATGCA CCGCAGCGCA ACGCGGGGAG GCAGACAAGG TATAGGGCGG
5521 CGCCTACAAT CCATGCCAAC CCGTTCCATG TGCTCGCCGA GCGGCGATAA ATCGCCGTGA
5581 CGATCAGCGG TCCAGTATC GAAGTTAGG TGGTAAGAGC CGCGAGCGAT CCTTGAAGCT
5641 GTCCTTGATG GTCGTATCT ACCTGCCTGG ACAGCATGGC CTGCAACGCG GGCATCCCGA
5701 TGCCGCCGGA AGCGAGAAGA ATCATAATGG GGAAGGCCAT CCAGCCTCGC GTCGCGAACG
5761 CCAGCAAGAC GTAGCCGAGC GCGTCGGCCG CCATGCCGGC GATAATGGCC TGCTTCTCGC
5821 CGAAACGTTT GGTGGCGGGA CCAGTGACGA AGGCTTGAGC GAGGGCGTGC AAGATTCCGA
5881 ATACCGCAAG CGACAGGCCG ATCATCGTCT CGCTCCAGCG AAAGCGGTCC TCGCCGAAAA
5941 TGACCCAGAG CGCTGCCGGC ACCTGTCTCA CGAGTTGCAT GATAAAGAAG ACAGTCATAA
6001 GTGCGGCGAC GATAGTCATG ACCCGCGCCC ACCGGAAGGA GCTGACTGGG TTGAAGGCTC
6061 TCAAGGGCAT CCGTGCATCG ACGTCTCTCC TTATGCGACT CCTGCATTAG GAAGCAGCCC
6121 AGTAGTAGGT TGAGGCCGTT GAGCACCGCC GCCGCAAGGA ATGGTGCATG CAAGGAGATG-

```

FIGURE 43C



123/240

6181 GCGCCCAACA GTCCCCCGGC CACGGGGCCT GCCACCATAC CCACGCCGAA ACAAGCGCTC  
6241 ATGAGCCCGA AGTGGCGAGC CCGA

FIGURE 43D

124/240  
pDEST24

## GST carboxy-fusion vector, T7 promoter

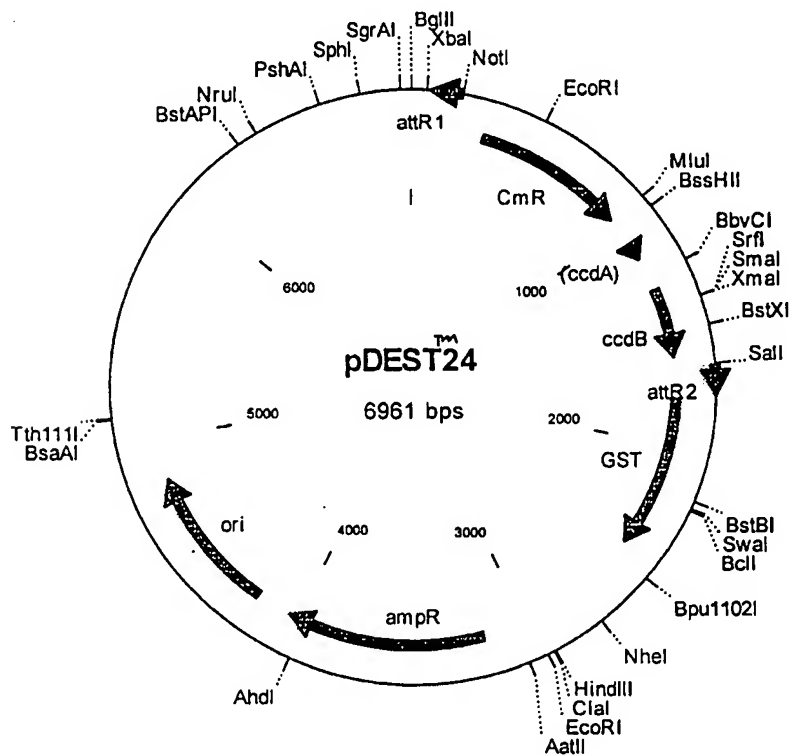
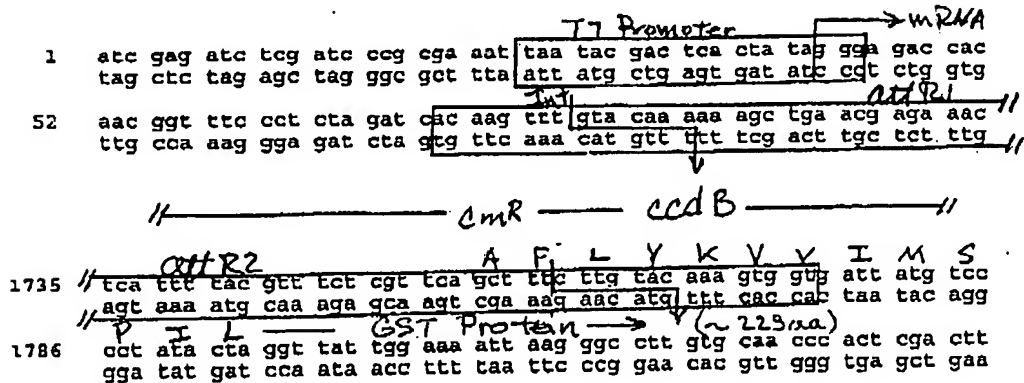


FIGURE 44A

125/240

## pDEST24 6961 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
195..71		attR1
304..963		CmR
1083..1167		inactivated ccdA
1305..1610		ccdB
1651..1775		attR2
1783..2451		GST
3181..4041		ampR
4190..4829		ori

1	ATCGAGATCT	CGATCCCGCG	AAATTAATAC	GACTCACTAT	AGGGAGACCA	CAACGGTTTC
61	CCTCTAGATC	ACAAGTTTGT	ACAAAAAGC	TGAACGAGAA	ACGTAAAATG	ATATAAATAT
121	CAATATATTA	AATTAGATTT	TGCATAAAAA	ACAGACTACA	TAATACTGTA	AAACACAACA
181	TATCCAGTCA	CTATGGCGGC	CGCATTAGGC	ACCCAGGCT	TTACACTTTA	TGCTTCCGGC
241	TCGTATAATG	TGTGGATTTT	GAGTTAGGAT	CCGGCGAGAT	TTTCAGGAGC	TAAGGAAGCT
301	AAAATGGAGA	AAAAAATCAC	TGGATATACC	ACCGTTGATA	TATCCCAATG	GCATCGTAAA
361	GAACATTTTG	AGGCATTTC	GTCAGTTGCT	CAATGTACCT	ATAACCAGAC	CGTTCAGCTG
421	GATATTACGG	CCTTTTAAA	GACCGTAAAG	AAAAATAAGC	ACAAGTTTTA	TCCGGCCTTT
481	ATTCACATTC	TTGCCCGCCT	GATGAATGCT	CATCCGGAAT	TCCGTATGGC	AATGAAAGAC
541	GGTGAGCTGG	TGATATGGGA	TAGTGTTCAC	CCTTGTAC	CCGTTTCCA	TGAGCAAAC
601	GAAACGTTTT	CATCGCTCTG	GAGTGAATAC	CACGACGATT	TCCGGCAGTT	TCTACACATA
661	TATTCGCAAG	ATGTGGCGTG	TTACGGTGAA	AACCTGGCCT	ATTCCCTTAA	AGGGTTTATT
721	GAGAATATGT	TTTTCGTCTC	AGCCAATCCC	TGGGTGAGTT	TCACCAGTTT	TGATTTAAAC
781	GTGGCCAATA	TGGACAACCT	CTTCGCCCC	GTTTTCACCA	TGGGCAAATA	TTATACGCAA
841	GGCGACAAGG	TGCTGATGCC	GCTGGCGATT	CAGGTCATC	ATGCCGCTG	TGATGGCTTC
901	CATGTCGGCA	GAATGCTTAA	TGAATTACAA	CAGTACTGCG	ATGAGTGGCA	GGGCGGGCG
961	TAAACGCGTG	GATCCGGCTT	ACTAAAAGCC	AGATAACAGT	ATGCGTATTT	GCGCGCTGAT
1021	TTTTGCGGTA	TAAGAATATA	TACTGATATG	TATACCCGAA	GTATGTCAAA	AAGAGGTGTG
1081	CTATGAAGCA	GCGTATTACA	GTGACAGTTG	ACAGCGACAG	CTATCAGTTG	CTCAAGGCAT
1141	ATATGATGTC	AATATCTCCG	GTCTGGTAA	CACAACCATG	CAGAATGAAG	CCCGTCTGCT
1201	GCGTGCCGAA	CGCTGGAAAG	CGGAAAATCA	GGAAGGGATG	GCTGAGGTCG	CCCGGTTTAT
1261	TGAAATGAAC	GGCTCTTTTG	CTGACGAGAA	CAGGGACTGG	TGAAATGCAG	TTTAAGGTTT
1321	ACACCTATAA	AAGAGAGAGC	CGTTATCGTC	TGTTTGTGGA	TGTACAGAGT	GATATTATTG
1381	ACACGCCCGG	GCGACGGATG	GTGATCCCC	TGGCCAGTGC	ACGCTGCTG	TCAGATAAAG
1441	TCTCCCGTGA	ACTTTACCCG	GTGGTGCATA	TCGGGGATGA	AAGCTGGCGC	ATGATGACCA
1501	CCGATATGGC	CAGTGTGCCG	GTCTCCGTTA	TCGGGGAAGA	AGTGGCTGAT	CTCAGCCACC
1561	GCGAAAATGA	CATCAAAAAC	GCCATTAAAC	TGATGTTCTG	GGGAATATAA	ATGTCAGGCT
1621	CCCTTATACA	CAGCCAGTCT	GCAGGTCGAC	CATAGTGACT	GGATATGTTG	TGTTTTACAG
1681	TATTATGTAG	TCTGTTTTTT	ATGCAAAATC	TAATTTAATA	TATTGATATT	TATATCATT
1741	TACGTTTCTC	GTTTACGCTT	CTGTACAAA	GTGGTGATTA	TGTTCCCTAT	ACTAGGTTAT
1801	TGGAAAATTA	AGGGCCTTGT	GCAACCCACT	CGACTTCTTT	TGGAATATCT	TGAAGAAAAA
1861	TATGAAGAGC	ATTTGTATGA	GCGCGATGAA	GGTGATAAAT	GGCGAAACAA	AAAGTTTGAA
1921	TTGGGTTTGG	AGTTTCCCAA	TCTTCCTTAT	TATATTGATG	GTGATGTTAA	ATTAACACAG
1981	TCTATGGCCA	TCATACGTTA	TATAGCTGAC	AAGCACAAAC	TGTTGGGTGG	TTGTCCAAAA
2041	GAGCGTGCG	AGATTTCAT	GCTTGAAGGA	GCGGTTTTGG	ATATTAGATA	CGGTGTTTCG
2101	AGAATTGCAT	ATAGTAAAGA	CTTTGAAACT	CTCAAAGTTG	ATTTTCTTAG	CAAGCTACCT
2161	GAAATGCTGA	AAATGTTTGA	AGATCGTTTA	TGTCATAAAA	CATATTTAAA	TGGTGATCAT
2221	GTAACCCATC	CTGACTTCAT	GTTGTATGAC	GCTCTTGATG	TTGTTTTATA	CATGGACCCA
2281	ATGTGCCTGG	ATGCGTTCCC	AAAATTAGTT	TGTTTTAAAA	AACGTATTGA	AGCTATCCCA
2341	CAAATTGATA	AGTACTTGAA	ATCCAGCAAG	TATATAGCAT	GGCCTTTGCA	GGGCTGGCAA
2401	GCCACGTTTG	GTGGTGGCGA	CCATCCTCCA	AAATCGGATC	TGGTTCCGCG	TCCATGGGGA
2461	TCCGGCTGCT	AACAAAGCCC	GAAAGGAAGC	TGAGTTGGCT	GCTGCCACCG	CTGAGCAATA
2521	ACTAGCATAA	CCCCTTGGGG	CCTCTAAACG	GGTCTTGAGG	GGTTTTTTGC	TGAAAGGAGG
2581	AACTATATCC	GGATATCCAC	AGGACGGGTG	TGGTCGCCAT	GATCGCGTGG	TCGATAGTGG
2641	CTCCAAGTAG	CGAAGCGAGC	AGGACTGGGC	GGCGGCCAAA	GCGGTCCGAC	AGTGCTCCGA-

FIGURE 44B

126/240

2701 GAACGGGTGC GCATAGAAAT TGCATCAACG CATATAGCGC TAGCAGCACG CCATAGTGAC  
2761 TGGCGATGCT GTCGGAATGG ACGATATCCC GCAAGAGGCC CGGCAGTACC GGCATAACCA  
2821 AGCCTATGCC TACAGCATCC AGGGTGACGG TGCCGAGGAT GACGATGAGC GCATTGTTAG  
2881 ATTTCATACA CGGTGCCTGA CTGCGTTAGC AATTTAACTG TGATAAACTA CCGCATTAATA  
2941 GCTTATCGAT GATAAGCTGT CAAACATGAG AATTCTTGAA GACGAAAGGG CCTCGTGATA  
3001 CGCCTATTTT TATAGTTAA TGTCATGATA ATAATGGTTT CTTAGACGTC AGGTGGCACT  
3061 TTTTCGGGAA ATGTGCGCGG AACCCCTATT TGTTTATTTT TCTAAATACA TTCAAATATG  
3121 TATCCGCTCA TGAGACAATA ACCCTGATAA ATGCTTCAAT AATATTGAAA AAGGAAGAGT  
3181 ATGAGTATTC AACATTTCCG TGTCGCCCTT ATTCCCTTTT TTGCGGCATT TTGCCCTCCT  
3241 GTTTTTGCTC ACCCAGAAAC GCTGGTGAAA GTAAAAGATG CTGAAGATCA GTTGGGTGCA  
3301 CGAGTGGGTT ACATCGAACT GGATCTCAAC AGCGGTAAGA TCCTTGAGAG TTTTCGCCCC  
3361 GAAGAACGTT TTCCAATGAT GAGCACTTTT AAAGTTCTGC TATGTGGCGC GGTATTATCC  
3421 CGTGTGACG CCGGGCAAGA GCAACTCGT CGCCGCATAC ACTATTCTCA GAATGACTTG  
3481 GTTGAGTACT CACCACTCAC AGAAAAGCAT CTTACGATG GCATGACAGT AAGAGAATTA  
3541 TGCAGTGCTG CCATAACCAT GAGTGATAAC ACTGCGGCCA ACTTACTTCT GACAACGATC  
3601 GGAGGACCGA AGGAGCTAAC CGCTTTTTTG CACAACATGG GGGATCATGT AACTCGCCTT  
3661 GATCGTTGGG AACCGGAGCT GAATGAAGCC ATACCAAACG ACGAGCGTGA CACCACGATG  
3721 CCTGCAGCAA TGGCAACAAC GTTGCGCAAA CTATTAACCTG GCGAACTACT TACTCTAGCT  
3781 TCCCGGCAAC AATTAATAGA CTGGATGGAG GCGGATAAAG TTGCAGGACC ACTTCTGCGC  
3841 TCGGCCCTTC CGGCTGGCTG GTTTATTGCT GATAAATCTG GAGCCGGTGA GCGTGGGTCT  
3901 CGCGGTATCA TTGCAGCACT GGGGCCAGAT GGTAAGCCCT CCCGTATCGT AGTATCTAC  
3961 ACGACGGGGA GTCAGGCAAC TATGGATGAA CGAAATAGAC AGATCGCTGA GATAGGTGCC  
4021 TCACTGATTA AGCATTGGTA ACTGTCAGAC CAAGTTTACT CATATATACT TTAGATTGAT  
4081 TTAAGACTTC ATTTTAAATT TAAAAGGATC TAGGTGAAGA TCCTTTTGA TAATCTCATG  
4141 ACCAAATCC CTTAACGTGA GTTTTCGTTT CACTGAGCGT CAGACCCCGT AGAAAAGATC  
4201 AAAGGATCTT CTTGAGATCC TTTTTTCTG CGCGTAATCT GCTGCTTGCA AACAAAAAAA  
4261 CCACCGCTAC CAGCGGTGGT TTGTTTGCCG GATCAAGAGC TACCAACTCT TTTTCCGAAG  
4321 GTAAGTGGT TCAGCAGAGC GCAGATACCA AATACTGTCC TTCTAGTGTA GCCGTAGTTA  
4381 GGCCACCACT TCAAGAACTC TGTAGCACCG CCTACATACC TCGCTCTGCT AATCCTGTTA  
4441 CCAGTGGCTG CTGCCAGTGG CGATAAGTCG TGTCTTACCG GGTGAGACTC AAGACGATAG  
4501 TTACCGGATA AGGCGCAGCG GTCGGGCTGA ACGGGGGGTT CGTGACACA GCCCAGCTTG  
4561 GAGCGAACGA CCTACACCGA ACTGAGATAC CTACAGCGTG AGCTATGAGA AAGCGCCACG  
4621 CTTCCCGAAG GGAGAAAGGC GGACAGGTAT CCGGTAAGCG GCAGGGTCGG AACAGGAGAG  
4681 CGCACGAGGG AGCTTCCAGG GGGAAACGCC TGGTATCTTT ATAGTCCTGT CGGGTTTCGC  
4741 CACCTCTGAC TTGAGCGTCG ATTTTGTGA TGCTCGTCAG GGGGGCGGAG CCTATGGA  
4801 AACGCCAGCA ACGCGGCCTT TTTACGGTTT CTGGCCTTTT GCTGGCCTTT TGCTACATG  
4861 TTCTTTCCTG CGTTATCCCC TGATTCTGTG GATAACCGTA TTACCGCCTT TGAGTGAGCT  
4921 GATACCGCTC GCCGCAGCCG AACGACCGAG CGCAGCGAGT CAGTGAGCGA GGAAGCGGAA  
4981 GAGCGCTGA TGCGGTATTT TCTCCTTACG CATCTGTGCG GTATTTTACA CCGCATATAT  
5041 GGTGCACTCT CAGTACAATC TGCTCTGATG CCGCATAGTT AAGCCAGTAT ACACCTCCGT  
5101 ATCGCTACGT GACTGGGTCA TGGCTGCGCC CCGACACCCG CCAACACCCG CTGACGCGCC  
5161 CTGACGGGCT TGTCTGCTCC CGGCATCCG TTACAGACAA GCTGTGACCG TCTCCGGGAG  
5221 TGGCATGTGT CAGAGGTTTT CACCGTCATC ACCGAAACGC GCGAGGCGAG TCGGGTAAAG  
5281 CTCATCAGCG TGGTCGTGAA GCGATTACCA GATGTCTGCC TGTTCATCCG CGTCCAGCTC  
5341 GTTGAGTTTC TCCAGAAGCG TTAATGTCTG GCTTCTGATA AAGCGGGCCA TGTTAAGGGC  
5401 GGTTTTTTCC TGTTTGGTCA CTGATGCCCT CGTGTAAGGG GGATTCTGT TCATGGGGGT  
5461 AATGATACCG ATGAAACGAG AGAGGATGCT CACGATACCG GTTACTGATG ATGAACATGC  
5521 CCGGTTACTG GAACGTTGTG AGGGTAAACA ACTGGCGGTA TGGATGCGGC GGGACCAGAG  
5581 AAAAACTACT CAGGGTCAAT GCCAGCGCTT CGTTAATACA GATGTAGGTG TTCCACAGGG  
5641 TAGCCAGCAG CATCCTGCGA TGCAGATCCG GAACATAATG GTGCAGGGCG CTGACTTCCG  
5701 CGTTTCCAGA CTTTACGAAA CACGGAAACC GAAGACCATT CATGTTGTTG CTCAGGTGCG  
5761 AGACGTTTTG CAGCAGCAGT CGCTTCACGT TCGCTCGCGT ATCGGTGATT CATCTGCTA  
5821 ACCAGTAAGG CAACCCCGCC AGCCTAGCCG GGTCTCAAC GACAGGAGCA CGATCATGCG  
5881 CACCCGTGGC CAGGACCCAA CGCTGCCCGA GATGCGCCCG GTGCGGCTGC TGGAGATGGC  
5941 GGACGCGATG GATATGTTCT GCCAAGGGTT GGTGTGCGCA TTCACAGTTC TCCGCAAGAA  
6001 TTGATTGGCT CCAATTCTTG GAGTGGTGAA TCCGTTAGCG AAGTGCCGCC GGCTTCCATT  
6061 CAGGTCGAGG TGGCCCGGCT CCATGCACCG CGACGCAACG CGGGGAGGCA GACAAGGTAT  
6121 AGGGCGGCGC CTACAATCCA TGCCAACCCG TTCCATGTGC TCGCCGAGGC GGCATAAATC

FIGURE 44C

127/240

6181 GCCGTGACGA TCAGCGGTCC AGTGATCGAA GTTAGGCTGG TAAGAGCCGC GAGCGATCCT  
6241 TGAAGCTGTC CCTGATGGTC GTCATCTACC TGCCTGGACA GCATGGCCTG CAACGCGGGC  
6301 ATCCCGATGC CGCCGGAAGC GAGAAGAATC ATAATGGGGA AGGCCATCCA GCCTCGCGTC  
6361 GCGAACGCCA GCAAGACGTA GCCCAGCGCG TCGGCCGCCA TGCCGGCGAT AATGGCCTGC  
6421 TTCTCGCCGA AACGTTTGGT GGCGGGACCA GTGACGAAGG CTTGAGCGAG GGC GTGCAAG  
6481 ATTCCGAATA CCGCAAGCGA CAGGCCGATC ATCGTCGCGC TCCAGCGAAA GCGGTCTCTG  
6541 CCGAAAATGA CCCAGAGCGC TGCCGGCACC TGTCTACGA GTTGCAATGAT AAAGAAGACA  
6601 GTCATAAGTG CGGCGACGAT AGTCATGCCC CGCGCCCACC GGAAGGAGCT GACTGGGTTG  
6661 AAGGCTCTCA AGGGCATCGG TCGATCGACG CTCTCCCTTA TCGCACTCCT GCATTAGGAA  
6721 GCAGCCCAGT AGTAGGTTGA GGCCGTTGAG CACCGCCGCC GCAAGGAATG GTGCATGCAA  
6781 GGAGATGGCG CCCAACAGTC CCCC GGCCAC GGGGCCTGCC ACCATACCCA CGCCGAAACA  
6841 AGCGCTCATG AGCCCGAAGT GGCAGGCCG ATCTTCCCCA TCGGTGATGT CGGCGATATA  
6901 GGCGCCAGCA ACCGCACCTG TGGCGCCGGT GATGCCGGCC ACGATGCGTC CGGCGTAGAG  
6961 G

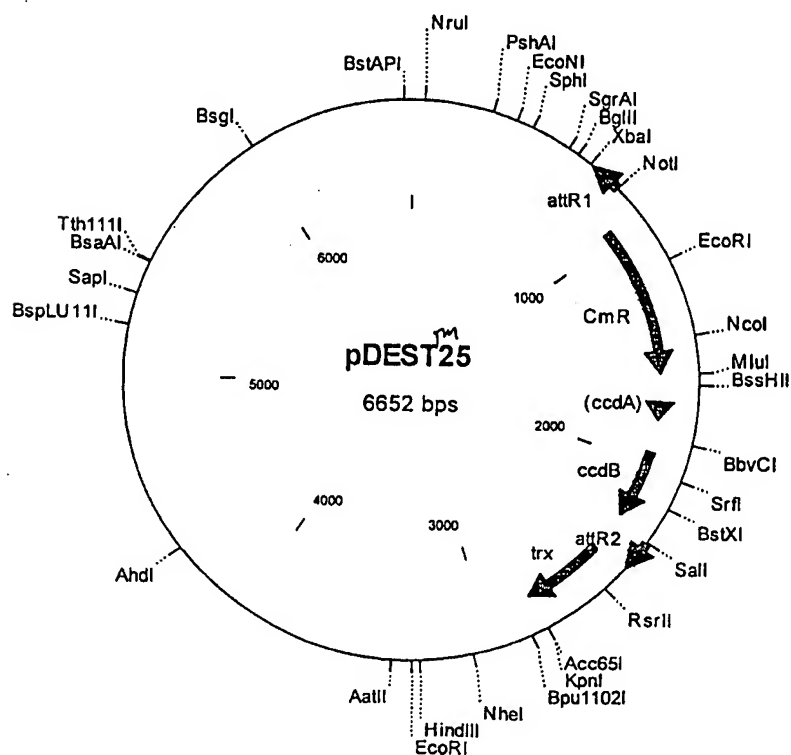
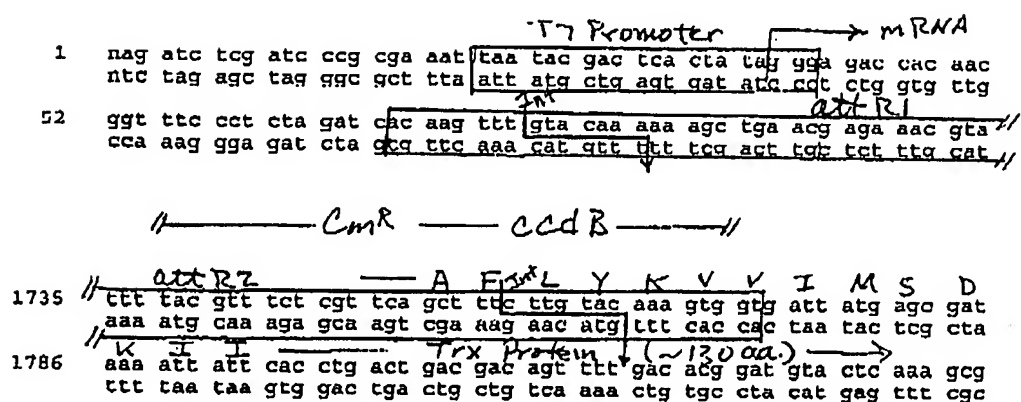
FIGURE 44D

128/240

FIGURE 45A

PDEST 25

Thioredoxin carboxy-fusion vector, T7 promoter



129/240

## pDEST25 6652 bp

<u>Location (Base Nos.)</u>	<u>Gene Encoded</u>
844..720	attR1
953..1612	CmR
1732..1816	inactivated ccdA
1954..2259	ccdB
2300..2424	attR2
2432..2794	trx

```

1  CCGGAAGCGA GAAGAATCAT AATGGGGAAG GCCATCCAGC CTCGCGTCGC GAACGCCAGC
61  AAGACGTAGC CCAGCGCGTC GGCCGCCATG CCGGCGATAA TGGCCTGCTT CTCGCCGAAA
121 CGTTTGGTGG CGGGACCAGT GACGAAGGCT TGAGCGAGGG CGTGCAAGAT TCCGAATACC
181 GCAAGCGACA GGCCGATCAT CGTCGCGCTC CAGCGAAAGC GGTCTCGCC GAAAATGACC
241 CAGAGCGCTG CCGGCACCTG TCCTACGAGT TGCATGATAA AGAAGACAGT CATAAGTGCG
301 GCGACGATAG TCATGCCCGG CGCCCACCGG AAGGAGCTGA CTGGGTTGAA GGCTCTCAAG
361 GGCATCGGTC GATCGACGCT CTCCCTTATG CGACTCCTGC ATTAGGAAGC AGCCAGTAG
421 TAGGTTGAGG CCGTTGAGCA CCGCCGCCGC AAGGAATGGT GCATGCAAGG AGATGGCGCC
481 CAACAGTCCC CCGGCCACGG GGCCTGCCAC CATACCCACG CCGAAACAAG CGCTCATGAG
541 CCCGAAGTGG CGAGCCCGAT CTTCCCCATC GGTGATGTCG GCGATATAGG CGCCAGCAAC
601 CGCACCTGTG GCGCCGCTGA TGCCGGCCAC GATGCGTCCG GCGTAGAGGA TCGAGATCTC
661 GATCCCGCGA AATTAATACG ACTCACTATA GGGAGACCAC AACGGTTTCC CTCTAGATCA
721 CAAGTTTGTA CAAAAAGCT GAACGAGAAA CGTAAATGA TATAAATATC AATATATTAA
781 ATTAGATTTT GCATAAAAAA CAGACTACAT AATACTGTAA AACACAACAT ATCCAGTCAC
841 TATGGCGGCC GCATTAGGCA CCCCAGGCTT TACACTTTAT GCTTCCGGCT CGTATAATGT
901 GTGGATTTTG AGTTAGGATC CGGCGAGATT TTCAGGAGCT AAGGAAGCTA AAATGGAGAA
961 AAAAATCACT GGATATACCA CCGTTGATAT ATCCCAATGG CATCGTAAAG AACATTTTGA
1021 GGCAATTCAG TCAGTTGCTC AATGTACCTA TAACCAGACC GTTCAGCTGG ATATTACGGC
1081 CTTTTTAAAG ACCGTAAAGA AAAATAAGCA CAAGTTTTAT CCGGCCTTTA TTCACATTCT
1141 TGCCCGCCTG ATGAATGCTC ATCCGGAATT CCGTATGGCA ATGAAAGACG GTGAGCTGGT
1201 GATATGGGAT AGTGTTACAC CTTGTTACAC CGTTTTCCAT GAGCAAACG AAACGTTTTT
1261 ATCGCTCTGG AGTGAATACC ACGACGATTT CCGGCAGTTT CTACACATAT ATTTCGAAGA
1321 TGTGGCGTGT TACGGTGAAA ACCTGGCCTA TTTCCCTAAA GGGTTTATG AGAATATGTT
1381 TTTCTGCTCA GCCAATCCCT GGGTGAGTTT CACCAGTTTT GATTTAAACG TGGCCAATAT
1441 GGACAACTTC TTCGCCCCCG TTTTCACCAT GGGCAAATAT TATACGCAAG GCGACAAGGT
1501 GCTGATGCCG CTGGCGATTC AGGTTTCATCA TGCCGTCGTG GATGGCTTCC ATGTCGGCAG
1561 AATGCTTAAT GAATTACAAC AGTACTGCGA TGAGTGCCAG GCGGGGCGCT AAACGCGTGG
1621 ATCCGGCTTA CTAAAAGCCA GATAACAGTA TCGGTATTG CCGCTGATT TTTGCGGTAT
1681 AAGAAATAT ACTGATATGT ATACCCGAAG TATGTCAAAA AGAGGTGTGC TATGAAGCAG
1741 CGTATTACAG TGACAGTTGA CAGCGACAGC TATCAGTTGC TCAAGGCATA TATGATGTCA
1801 ATATCTCCGG TCTGGTAAGC ACAACCATGC AGAATGAAGC CCGTCGCTCG CGTGCCGAAC
1861 GCTGGAAGC GGAATAATCAG GAAGGGATGG CTGAGGTCGC CCGGTTTTAT GAAATGAACG
1921 GCTCTTTTGC TGACGAGAAC AGGGACTGGT GAAATGCAGT TTAAGGTTTA CACCTATAAA
1981 AGAGAGAGCC GTTATCGTCT GTTTGTGGAT GTACAGAGTG ATATTATTGA CACGCCCGGG
2041 CGACGGATGG TGATCCCCCT GGCCAGTGCA CGTCTGCTGT CAGATAAAGT CTCCCGTGAA
2101 CTTTACCCGG TGGTGATAT CCGGGATGAA AGCTGGCGCA TGATGACCAC CGATATGGCC
2161 AGTGTGCCGG TCTCCGTTAT CCGGGAAGAA GTGGCTGATC TCAGCCACCG CGAAAATGAC
2221 ATCAAAAACG CCATTAACCT GATGTTCTGG GGAATATAAA TGTCAGGCTC CCTTATACAC
2281 AGCCAGTCTG CAGGTGCGAC ATAGTGACTG GATATGTTGT GTTTTACAGT ATTATGTAGT
2341 CTGTTTTTTA TGCAAAATCT AATTTAATAT ATTGATATTT ATATCATTTT ACGTTTCTCG
2401 TTCAGCTTTC TTGTACAAAG TGGTGATTAT GAGCGATAAA ATTATTCACC TGACTAGCGA
2461 CAGTTTTGAC ACGGATGTAC TCAAAGCGGA CCGGGCGATC CTCGTCGATT TCTGGGCAGA
2521 GTGGTGCGGT CCGTGCAAAA TGATCGCCCC GATTCTGGAT GAAATCGCTG ACGAATATCA
2581 GGGCAAACG ACCGTTGCAA AACTGAACAT CGATCAAAAC CCTGGCACTG CGCCGAAATA
2641 TGGCATCCGT GGTATCCCGA CTCTGCTGCT GTTCAAAAAC GGTGAAGTGG CGGCAACCAA
2701 AGTGGGTGCA CTGTCTAAAG GTCAGTTGAA AGAGTTCTCT GACGCTAACC TGGCCGGTTC
2761 TGGTCTGGT GATGACGATG ACAAGGTACC CCGGGATCGA TCCGCTGCT AACAAAGCCC

```

Figure 45B

130/240

2821 GAAAGGAAGC TGAGTTGGCT GCTGCCACCG CTGAGCAATA ACTAGCATAA CCCCTTGGGG  
2881 CCTCTAAACG GGTCTTGAGG GGTTTTTTGC TGAAAGGAGG AACTATATCC GGATATCCAC  
2941 AGGACGGGTG TGGTCGCCAT GATCGCGTAG TCGATAGTGG CTCCAAGTAG CGAAGCGAGC  
3001 AGGACTGGGC GCGGCCCAA GCGGTGCGAC AGTGCTCCGA GAACGGGTGC GCATAGAAAT  
3061 TGCATCAACG CATATAGCGC TAGCAGCAGC CCATAGTGAC TGGCGATGCT GTCGGAATGG  
3121 ACGATATCCC GCAAGAGGCC CGGCAGTACC GGCATAACCA AGCCTATGCC TACAGCATCC  
3181 AGGGTGACGG TGCCGAGGAT GACGATGAGC GCATTGTTAG ATTTTCATACA CGGTGCCTGA  
3241 CTGCGTTAGC AATTTAACTG TGATAAACTA CCGCATTAAA GCTTATCGAT GATAAGCTGT  
3301 CAAACATGAG AATTCTTGAA GACGAAAGGG CCTCGTGATA CGCCTATTTT TATAGGTTAA  
3361 TGTCATGATA ATAATGGTTT CTTAGACGTC AGGTGGCACT TTTCCGGGAA ATGTGCGCGG  
3421 AACCCCTATT TGTTTATTTT TCTAAATACA TTCAAATATG TATCCGCTCA TGAGACAATA  
3481 ACCCTGATAA ATGCTTCAAT AATATTGAAA AAGGAAGAGT ATGAGTATTC AACATTTCCG  
3541 TGTCGCCCTT ATTCCCTTTT TTGCGGCATT TTGCCTTCCT GTTTTGTGCT ACCCAGAAAC  
3601 GCTGGTGAAA GTAAAAGATG CTGAAGATCA GTTGGGTGCA CGAGTGGGT ACATCGAAT  
3661 GGATCTCAAC AGCGGTAAGA TCCTTGAGAG TTTTCGCCCC GAAGAACGTT TTCCAATGAT  
3721 CAGCACTTTT AAAGTTCTGC TATGTGGCGC GGTATTATCC CGTGTGACG CCGGGCAAGA  
3781 GCAACTCGGT CGCCGCATAC ACTATTCTCA GAATGACTTG GTTGAGTACT CACCAGTCAC  
3841 AGAAAAGCAT CTTACGGATG GCATGACAGT AAGAGAATTA TGCAGTGCTG CCATAACCAT  
3901 GAGTGATAAC ACTGCGGCCA ACTTACTTCT GACAACGATC GGAGGACCGA AGGAGCTAAC  
3961 CGTTTTTTTG CACAACATGG GGGATCATGT AACTCGCCTT GATCGTTGGG AACCGGAGCT  
4021 GAATGAAGCC ATACCAAACG ACGAGCGTGA CACCACGATG CCTGCAGCAA TGGCAACAAC  
4081 GTTGCGCAAA CTATTAAGTG GCGAACTACT TACTCTAGCT TCCCGGCAAC AATTAATAGA  
4141 CTGGATGGAG GCGGATAAAG TTGCAGGACC ACTTCTGCGC TCGGCCCTTC CGGCTGGCTG  
4201 GTTTATTGCT GATAAATCTG GAGCCGGTGA GCGTGGGTCT CGCGGTATCA TTGCAGCACT  
4261 GGGGCCAGAT GGTAAGCCCT CCCGTATCGT AGTTATCTAC ACGACGGGGA GTCAGGCAAC  
4321 TATGGATGAA CGAAATAGAC AGATCGCTGA GATAGGTGCC TCACTGATTA AGCATTGGTA  
4381 ACTGTCAGAC CAAGTTTACT CATATATACT TTAGATTGAT TTAAAACTTC ATTTTAAAT  
4441 TAAAAGGATC TAGGTGAAGA TCCTTTTGA TAATCTCATG ACCAAAATCC CTTAACGTGA  
4501 GTTTTCGTTT CACTGAGCGT CAGACCCCGT AGAAAAGATC AAAGGATCTT CTGAGATCC  
4561 TTTTTTTCTG CGCGTAATCT GCTGCTTGCA AACAAAAAAA CCACCGCTAC CAGCGGTGGT  
4621 TTGTTTGCCG GATCAAGAGC TACCAACTCT TTTTCCGAAG GTAAGTGGCT TCAGCAGAGC  
4681 GCAGATACCA AATACTGTCC TTCTAGTGTA GCCGTAGTTA GGCCACCACT TCAAGAACTC  
4741 TGTAGCACCG CCTACATACC TCGCTCTGCT AATCCTGTTA CCAGTGGCTG CTGCCAGTGG  
4801 CGATAAGTCG TGTCTTACCG GGTGAGACTC AAGACGATAG TTACCGGATA AGGCGCAGCG  
4861 GTCGGGCTGA ACGGGGGGTT CGTGACACA GCCCAGCTTG GAGCGAACGA CCTACACCGA  
4921 ACTGAGATAC CTACAGCGTG AGCTATGAGA AAGCGCCACG CTTCCCGAAG GGAGAAAGGC  
4981 GGACAGGTAT CCGGTAAGCG GCAGGTCGG AACAGGAGAG CGCAGGAGG AGCTTCCAGG  
5041 GGGAAACGCC TGGTATCTT ATAGTCTGT CGGGTTTCGC CACCTCTGAC TTGAGCGTCG  
5101 ATTTTGTGTA TGCTCGTCAG GGGGGCGGAG CCTATGAAA AACGCCAGCA ACGCGGCCTT  
5161 TTTACGGTTC CTGGCCTTTT GCTGGCCTTT TGCTCACATG TTCTTTCTG CGTTATCCCC  
5221 TGATTCTGTG GATAACCGTA TTACCGCCTT TGAGTGAGCT GATACCGCTC GCCCGAGCCG  
5281 AACGACCGAG CGCAGCGAGT CAGTGAGCGA GGAAGCGGAA GAGCGCTGA TGCGGTATTT  
5341 TCTCCTTACG CATCTGTGCG GTATTTTACA CCGCATATAT GGTGCACTCT CAGTACAATC  
5401 TGCTCTGATG CCGCATAGTT AAGCCAGTAT ACACCTCCGT ATCGCTACGT GACTGGGTCA  
5461 TGGCTGCGCC CCGACACCCG CCAACACCCG CTGACGCGCC CTGACGGGCT TGTCTGCTCC  
5521 CGGCATCCGC TTACAGACAA GCTGTGACCG TCTCCGGGAG CTGCATGTGT CAGAGGTTTT  
5581 CACCGTCATC ACCGAAACGC GCGAGGCAGC TGCGGTAAAG CTCATCAGCG TGGTCGTGAA  
5641 GCGATTACCA GATGTCTGCC TGTTTCATCCG CGTCCAGCTC GTTGAGTTTC TCCAGAAGCG  
5701 TTAATGTCTG GCTTCTGATA AAGCGGGCCA TGTTAAGGGC GGTTTTTTCC TGTTTGGTCA  
5761 CTGATGCCTC CGTGTAAGGG GGATTTCTGT TCATGGGGGT AATGATACCG ATGAAACGAG  
5821 AGAGGATGCT CACGATACGG GTTACTGATG ATGAACATGC CCGGTTACTG GAACGTTGTG  
5881 AGGGTAAACA ACTGGCGGTA TGGATGCGGC GGGACCAGAG AAAAATCACT CAGGGTCAAT  
5941 GCCAGCGCTT CGTTAATACA GATGTAGGTG TTCCACAGGG TAGCCAGCAG CATCCTGCGA  
6001 TGCAGATCCG GAACATAATG GTGCAGGCG CTGACTTCCG CGTTTCCAGA CTTTACGAAA  
6061 CACGGAACCC GAAGACCATT CATGTTGTTG CTCAGGTCGC AGACGTTTTG CAGCAGCAGT  
6121 CGCTTCACGT TCGCTCGCGT ATCGGTGATT CATTTCTGCTA ACCAGTAAGG CAACCCCGCC  
6181 AGCCTAGCCG GGTCCCTCAAC GACAGGAGCA CGATCATGCG CACCCGTGGC CAGGACCCAA  
6241 CGCTGCCCGA GATGCGCCGC GTGCGGCTGC TGGAGATGGC GGACGCGATG GATATGTTCT

FIGURE 45C



6301 GCCAAGGGTT GGTTTGC GCA TTCACAGTTC TCCGCAAGAA TTGATTGGCT CCAATTCTTG  
6361 GAGTGGTGAA TCCGTTAGCG AGGTGCCGCC GGCTTCCATT CAGGTCGAGG TGGCCCGGCT  
6421 CCATGCACCG CGACGCAACG CGGGGAGGCA GACAAGGTAT AGGGCGGCGC CTACAATCCA  
6481 TGCCAACCCG TTCCATGTGC TCGCCGAGGC GGCATAAATC GCCGTGACGA TCAGCGGTCC  
6541 AGTGATCGAA GTTAGGCTGG TAAGAGCCGC GAGCGATCCT TGAAGCTGTC CCTGATGGTC  
6601 GTCATCTACC TGCCTGGACA GCATGGCCTG CAACGCGGGC ATCCCGATGC CG

132/240

FIGURE 46A

# pDEST26 His6 Amino Fusion in pCMV Sport-neo Vector

```

600   ttg acg tca atg gga gtt tgt ttt ggc acc aaa atc aac ggg act ttc' caa
      aac tgc agt tac cct caa aca aaa ccg tgg ttt tag ttg ccc tga aag gtt

651   aat gtc gta aca act ccg ccc oat tga cgc aaa tgg gcg gta ggc gtg tac
      tta cag cat tgt tga ggc ggg gta act gcg ttt acc cgc cat ccg cac atg

702   // ggt ggg agg tct ata taa goa gag etc gtt tag tga acc gtc aga tgg cct
      //cca ccc tcc aga tat att cgt etc gag caa atc act tgg cag tct agc gga

753   gga gac gcc atc cac gct gtt ttg acc tcc ata gaa gac acc ggg acc gat
      cct ctg cgg tag gtg cga caa aac tgg agg tat ctt ctg tgg ccc tgg cta

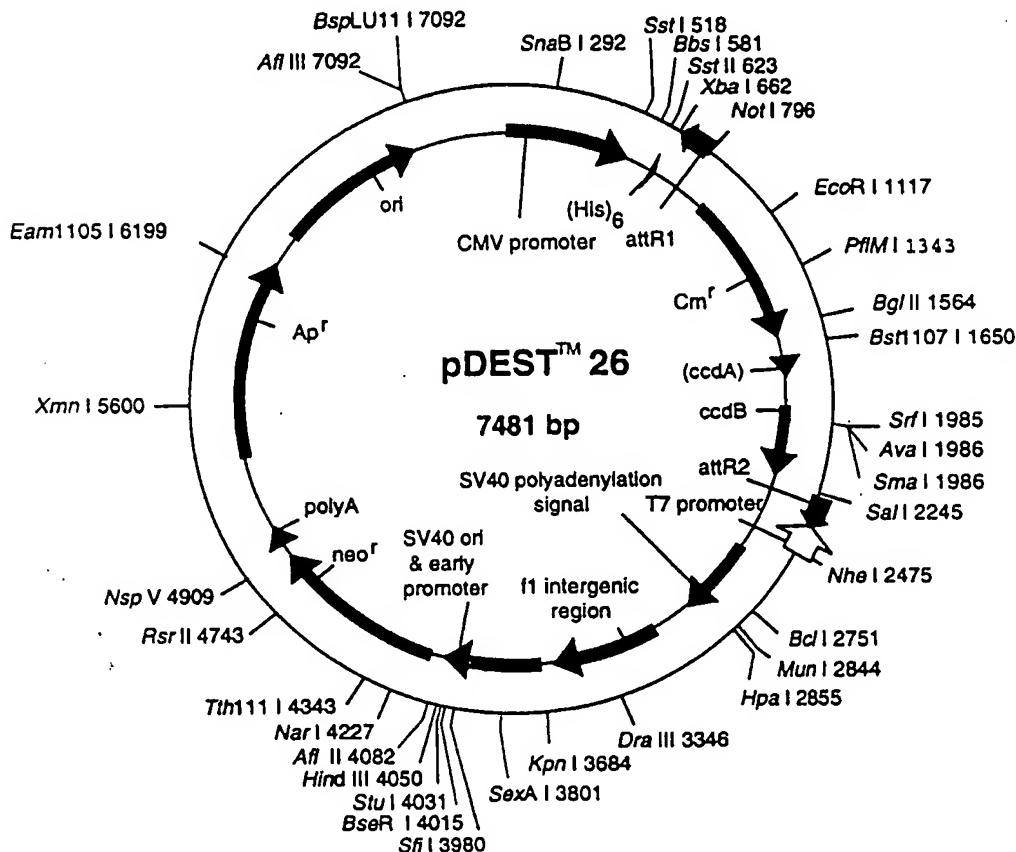
804   cca gcc tcc gga ctc tag cct agg ccg cgg acc atg gcg tac tac cat cac
      ggt cgg agg cct gag atc gga tcc gcc gcc tgg tac cgc atg atg gta gtc

855   H H H H S R S T S H V K K A
      cat cac cat cac tct aga tca aca agt ttg tac aaa aaa gct gaa cga gaa
      gta gtg gta gtg aga tct agt tgt tca aac atg ttt ttt cga ctt gct ctt
  
```

CMV Promoter → mRNA

Start Transl →

Int V



## pDEST26 7481 bp

<u>Location (Base Nos.)</u>	<u>Gene Encoded</u>
492..509	his6
619..519	attR1
752..1411	CmR
1531..1615	inactivated ccdA
1753..2058	ccdB
2099..2223	attR2
2409..2771	SV40 polyA
2966..3421	f1 intergenic region
3485..3903	SV40 promoter
3948..4742	neo
4806..4854	polyA
5265..6125	Apr
6274..6913	ori
7344..385	CMV promoter

```

1 GTAAACTGCC CACTTGGCAG TACATCAAGT GTATCATATG CCAAGTACGC CCCCTATTGA
61 CGTCAATGAC GGTAAATGGC CCGCCTGGCA TTATGCCCAG TACATGACCT TATGGGACTT
121 TCCTACTTGG CAGTACATCT ACGTATTAGT CATCGCTATT ACCATGGTGA TGC GGTTTTG
181 GCAGTACATC AATGGGCGTG GATAGCGGTT TGACTCACGG GGATTTCCAA GTCTCCACCC
241 CATTGACGTC AATGGGAGTT TGTTTTGGCA CCAAAATCAA CGGGACTTTC CAAAATGTCTG
301 TAACCAACTCC GCCCCATTGA CGCAAATGGG CGGTAGGCGT GTACGGTGGG AGGTCTATAT
361 AAGCAGAGCT CGTTTAGTGA ACCGTCAGAT CGCCTGGAGA CGCCATCCAC GCTGTTTTGA
421 CCTCCATAGA AGACACCGGG ACCGATCCAG CCTCCGGACT CTAGCCTAGG CCGCGGACCA
481 TGGCGTACTA CCATCACCAT CACCATCACT CTAGATCAAC AAGTTTGTAC AAAAAAGCTG
541 AACGAGAAAC GTAAATGAT ATAAATATCA ATATATTAA TTAGATTTTG CATAAAAAAC
601 AGACTACATA ATACTGTAAA ACACAACATA TCCAGTCACT ATGGCGGCCG CATTAGGCAC
661 CCCAGGCTTT ACACCTTTATG CTTCCGGCTC GTATAATGTG TGGATTTTGA GTTAGGATCC
721 GCGGAGATTT TCAGGAGCTA AGGAAGCTAA AATGGAGAAA AAAATCACTG GATATACCAC
781 CGTTGATATA TCCCAATGGC ATCGTAAAGA ACATTTTGAG GCATTTTCAGT CAGTTGCTCA
841 ATGTACCTAT AACCAGACCG TTCAGCTGGA TATTACGGCC TTTTAAAGA CCGTAAAGAA
901 AAATAAGCAC AAGTTTTATC CGGCCTTTAT TCACATTCTT GCCCGCTGA TGAATGCTCA
961 TCCGGAATTC CGTATGGCAA TGAAAGACGG TGAGCTGGTG ATATGGGATA GTGTTCACCC
1021 TTGTTACACC GTTTTCCATG AGCAAACGTA AACGTTTTCA TCGCTCTGGA GTGAATACCA
1081 CGACGATTTT CGGCAGTTTC TACACATATA TTCGCAAGAT GTGGCGTGT TACGGTGAAAA
1141 CCTGGCCTAT TTCCCTAAAG GGTTTATTTA GAATATGTTT TTCGTCTCAG CCAATCCCTG
1201 GGTGAGTTTC ACCAGTTTTG ATTTAAACGT GGCCAATATG GACAACTTCT TCGCCCCCGT
1261 TTTCAACATG GGCAAATATT ATACGCAAGG CGACAAGGTG CTGATGCCGC TGGCGATTCA
1321 GGTTTCATCAT GCGTCTGTG ATGGCTTCCA TGTCGGCAGA ATGCTTAATG AATTACAACA
1381 GTACTGCGAT GAGTGGCAGG GCGGGGCGTA AAGATCTGGA TCCGGCTTAC TAAAAGCCAG
1441 ATAACAGTAT GCGTATTTGC GCGCTGATTT TTGCGGTATA AGAATATATA CTGATATGTA
1501 TACCCGAAGT ATGTCAAAAA GAGGTGTGCT ATGAAGCAGC GTATTACAGT GACAGTTGAC
1561 AGCGACAGCT ATCAGTTGCT CAAGGCATAT ATGATGTCAA TATCTCCGGT CTGGTAAGCA
1621 CAACCATGCA GAATGAAGCC CGTCGTCTGC GTGCCGAACG CTGGAAAGCG GAAAATCAGG
1681 AAGGGATGGC TGAGGTCGCC CGGTTTATTG AAATGAACGG CTCTTTTGCT GACGAGAACA
1741 GGGACTGGTG AAATGCAGTT TAAGGTTTAC ACCTATAAAA GAGAGAGCCG TTATCGTCTG
1801 TTTGTGGATG TACAGAGTGA TATTATTGAC ACGCCCGGGC GACGGATGGT GATCCCCCTG
1861 GCCAGTGCAC GTCTGCTGTC AGATAAAGTC TCCCGTGAAC TTTACCCGGT GGTGCATATC
1921 GGGGATGAAA GCTGGCGCAT GATGACCACC GATATGGCCA GTGTGCCGGT CTCCGTTATC
1981 GGGGAAGAAG TGGCTGATCT CAGCCACCGC GAAAATGACA TCAAAAACGC CATTAACTCG
2041 ATGTTCTGGG GAATATAAAT GTCAGGCTCC CTTATACACA GCCAGTCTGC AGGTGACCA
2101 TAGTGACTGG ATATGTTGTG TTTTACAGTA TTATGTAGTC TGTTTTTTAT GCAAAATCTA
2161 ATTTAATATA TTGATATTTA TATCATTTTA CGTTTCTCGT TCAGCTTTCT TGTACAAAGT
2221 GGTTGATCGC GTGCATGCGA CGTCATAGCT CTCTCCCTAT AGTGAGTCGT ATTATAAGCT
2281 AGGCACTGGC CGTCGTTTTA CAACGTCGTG ACTGGGAAAA CTGCTAGCTT GGGATCTTTG --

```

2341 TGAAGGAACC TTACTTCTGT GGTGTGACAT AATTGGACAA ACTACCTACA GAGATTTAAA  
2401 GCTCTAAGGT AAATATAAAA TTTTAAAGTG TATAATGTGT TAAACTAGCT GCATATGCTT  
2461 GCTGCTTGAG AGTTTTGCTT ACTGAGTATG ATTTATGAAA ATATTATACA CAGGAGCTAG  
2521 TGATTCTAAT TGTTTGTTGA TTTTAGATTC ACAGTCCCAA GGCTCATTTT AGGCCCTCA  
2581 GTCCTCACAG TCTGTTTCATG ATCATAATCA GCCATACCAC ATTTGTAGAG GTTTTACTTG  
2641 CTTTAAAAAA CCTCCACAC CTCCCCCTGA ACCTGAAACA TAAAATGAAT GCAATTGTTG  
2701 TTGTTAACTT GTTTATTGCA GCTTATAATG GTTACAAATA AAGCAATAGC ATCACAAAT  
2761 TCACAAATAA AGCATTTTTT TCACTGCATT CTAGTTGTGG TTTGTCCAAA CTCATCAATG  
2821 TATCTTATCA TGTCTGGATC GATCCTGCAT TAATGAATCG GCCAACGCGC GGGGAGAGGC  
2881 GGTGTCGTA TTGGCTGGCG TAATAGCGAA GAGGCCCGCA CCGATCGCCC TTCCCAACAG  
2941 TTGCGCAGCC TGAATGGCGA ATGGGACGCG CCCTGTAGCG GCGCATTAAG CGCGCGGGT  
3001 GTGGTGGTTA CGCGCAGCGT GACCGCTACA CTTGCCAGCG CCCTAGCGCC CGCTCCTTC  
3061 GCTTCTTCC CTTCCTTCT CTCCACGTTT GCGGCTTTC CCCGTCAAGC TCTAAATCGG  
3121 GGGCTCCCTT TAGGGTCCG ATTTAGTGCT TTACGGCACC TCGACCCCAA AAAACTTGAT  
3181 TAGGGTGATG GTTCACGTAG TGGGCCATCG CCCTGATAGA CGGTTTTTCG CCCTTTGACG  
3241 TTGGAGTCCA CGTTCTTTAA TAGTGGACTC TTGTTCCAAA CTGGAACAAC ACTCAACCTT  
3301 ATCTCGGTCT ATTCTTTTGA TTTATAAGGG ATTTTGCCGA TTTGCGCCTA TTGGTTAAAA  
3361 AATGAGCTGA TTTAACAAAT ATTTAACGCG AATTTTAAACA AAATATTAAC GTTTACAATT  
3421 TCGCCTGATG CGGTATTTTC TCCTTACGCA TCTGTGCGGT ATTTACACCC GCATACGCGG  
3481 ATCTGCGCAG CACCATGGCC TGAATAAACC TCTGAAAGAG GAACTTGGTT AGGTACCTTC  
3541 TGAGGCGGAA AGAACGAGCT GTGGAATGTG TGTCAGTTAG GGTGTGGAAG GTCCCGAGC  
3601 TCCCGCAGC GCAGAAGTAT GCAAAGCATG CATCTCAATT AGTCAGCAAC CAGGTGTGGA  
3661 AAGTCCCCAG GCTCCCCAGC AGGCAGAAGT ATGCAAAGCA TGCATCTCAA TTAGTCAGCA  
3721 ACCATAGTCC CGCCCTAAC TCCGCCATC CCGCCCTAA CTCCGCCAG TTCCGCCCAT  
3781 TCTCCGCCCC ATGGCTGACT AATTTTTTTT ATTTATGCAG AGGCCGAGGC CGCCTCGGCC  
3841 TCTGAGCTAT TCCAGAAGTA GTGAGGAGGC TTTTTTGAG GCCTAGGCTT TTGCAAAAAG  
3901 CTTGATTCTT CTGACACAAC AGTCTCGAAC TTAAGGCTAG AGCCACCATG ATTGAACAAG  
3961 ATGGATTGCA CGCAGTTCT CCGGCCGCTT GGGTGGAGAG GCTATTCGGC TATGACTGGG  
4021 CACAACAGAC AATCGGCTGC TCTGATGCCG CCGTGTTCG GCTGTACGCG CAGGGGCGCC  
4081 CGGTTCTTTT TGTCAAGACC GACCTGTCCG GTGCCCTGAA TGAAGTGCAG GACGAGGCAG  
4141 CGCGGCTATC GTGGCTGGCC ACGACGGGCG TTCCTTGCGC AGCTGTGCTC GACGTTGTCA  
4201 CTGAAGCGGG AAGGGACTGG CTGCTATTGG GCGAAGTGCC GGGGCGAGGAT CTCCTGTCTC  
4261 CTCACCTTGC TCCTGCCGAG AAAGTATCCA TCATGGCTGA TGCAATGCGG CGGCTGCATA  
4321 CGCTTGATCC GGCTACCTGC CCATTGACG ACCAAGCGAA ACATCGCATC GAGCGAGCAC  
4381 GTACTCGGAT GGAAGCCGGT CTTGTGATC AGGATGATCT GGACGAAGAG CATCAGGGGC  
4441 TCGCGCCAGC CGAACTGTTT GCCAGGCTCA AGGCGCGCAT GCGCGACGGC GAGGATCTCG  
4501 TCGTGACCCA TGGCGATGCC TGCTTGCCGA ATATCATGGT GGAAAATGGC CGCTTTTCTG  
4561 GATTTCATCGA CTGTGGCCGG CTGGGTGTGG CGGACCGCTA TCAGGACATA GCGTTGGCTA  
4621 CCCGTGATAT TGCTGAAGAG CTTGGCGGCG AATGGGCTGA CCGCTTCTC GTGCTTTACG  
4681 GTATCGCCGC TCCCGATTCT CAGCGCATCG CCTTCTATCG CCTTCTTGAC GAGTTCTTCT  
4741 GAGCGGGACT CTGGGGTTCG AAATGACCGA CCAAGCGAGC CCCAACCTGC CATCAGCATG  
4801 GCCGCAATAA AATATCTTTA TTTTCATTAC ATCTGTGTGT TGGTTTTTGT TGTGAATCGA  
4861 TAGCGATAAG GATCCGCGTA TGGTGACATC TCAGTACAAT CTGCTCTGAT GCCGCATAGT  
4921 TAAGCCAGCC CCGACACCCG CCAACACCCG CTGACGCGCC CTGACGGGCT TGTCTGCTCC  
4981 CGGCATCCGC TTACAGACAA GCTGTGACCG TCTCCGGGAG CTGCATGTGT CAGAGGTTTT  
5041 CACCGTCATC ACCGAAACGC GCGAGACGAA AGGGCCTCGT GATACGCTA TTTTATAGG  
5101 TTAATGTCAT GATAATAATG GTTTCTTAGA CGTCAGGTGG CACTTTTCGG GGAAATGTGC  
5161 GCGGAACCCC TATTGTGTTA TTTTCTAAA TACATTCAA TATGTATCCG CTCATGAGAC  
5221 AATAACCTTG ATAAATGCTT CAATAATATT GAAAAAGGAA GAGTATGAGT ATTCACATT  
5281 TCCGTGTCGC CCTTATTCCT TTTTTGCGG CATTTGCTT TCCTGTTTTT GCTCAGCCAG  
5341 AAACGCTGGT GAAAGTAAAA GATGCTGAAG ATCAGTTGGG TGCACGAGTG GGTACATCG  
5401 AACTGGATCT CAACAGCGGT AAGATCCTTG AGAGTTTTCG CCGGAAGAA CGTTTTCCAA  
5461 TGATGAGCAC TTTTAAAGTT CTGCTATGTG GCGCGGTATT ATCCCGTATT GACGCCGGG  
5521 AAGAGCAACT CGGTGCGCCG ATACACTATT CTCAGAATGA CTTGGTTGAG TACTCACCAG  
5581 TCACAGAAAA GCATCTTACG GATGGCATGA CAGTAAGAGA ATTATGCAGT GCTGCCATAA  
5641 CCATGAGTGA TAACACTGCG GCCAACTTAC TTCTGACAAC GATCGGAGGA CCGAAGGAGC  
5701 TAACCGCTTT TTTGCACAAC ATGGGGGATC ATGTAACCTG CCTTGATCGT TGGGAACCGG  
5761 AGCTGAATGA AGCCATACCA AACGACGAGC GTGACACCAC GATGCCTGTA GCAATGGCAA -

5821 CAACGTTGCG CAAACTATTA ACTGGCGAAC TACTTACTCT AGCTTCCCGG CAACAATTAA  
5881 TAGACTGGAT GGAGGCGGAT AAAGTTGCAG GACCACTTCT GCGCTCGGCC CTTCGGCTG  
5941 GCTGGTTTAT TGCTGATAAA TCTGGAGCCG GTGAGCGTGG GTCTCGCGGT ATCATTGCAG  
6001 CACTGGGGCC AGATGGTAAG CCTCCCGTA TCGTAGTTAT CTACACGACG GGGAGTCAGG  
6061 CAACTATGGA TGAACGAAAT AGACAGATCG CTGAGATAGG TGCCTCACTG ATTAAGCATT  
6121 GGTAAGTGT AGACCAAGTT TACTCATATA TACTTTAGAT TGATTAAAA CTTCATTTTT  
6181 AATTTAAAAG GATCTAGGTG AAGATCCTTT TTGATAATCT CATGACCAA ATCCCTTAAC  
6241 GTGAGTTTTT GTTCCACTGA GCGTCAGACC CCGTAGAAAA GATCAAAGGA TCTTCTTGAG  
6301 ATCCTTTTTT TCTGCGCGTA ATCTGCTGCT TGCAAACAAA AAAACCACCG CTACCAGCGG  
6361 TGGTTTGTGT GCCGGATCAA GAGCTACCAA CTCTTTTCC GAAGGTAAGT GGCTTCAGCA  
6421 GAGCGCAGAT ACCAAATACT GTCCTTCTAG TGTAGCCGTA GTTAGGCCAC CACTTCAAGA  
6481 ACTCTGTAGC ACCGCCTACA TACCTCGCTC TGCTAATCCT GTTACCAGTG GCTGCTGCCA  
6541 GTGGCGATAA GTCGTGTCTT ACCGGGTTGG ACTCAAGACG ATAGTTACCG GATAAGGCGC  
6601 AGCGGTCGGG CTGAACGGGG GGTTCGTGCA CACAGCCAG CTTGGAGCGA ACGACCTACA  
6661 CCGAACTGAG ATACCTACAG CGTGAGCATT GAGAAAGCGC CACGCTTCCC GAAGGGAGAA  
6721 AGGCGGACAG GTATCCGGTA AGCGGCAGGG TCGGAACAGG AGAGCGCACG AGGGAGCTTC  
6781 CAGGGGGAAA CGCCTGGTAT CTTTATAGTC CTGTCGGGTT TCGCCACCTC TGAAGGAGC  
6841 GTCGATTTTT GTGATGCTCG TCAGGGGGGG GGAGCCTATG GAAAAACGCC AGCAACGCGG  
6901 CCTTTTACG GTTCCTGGCC TTTTGCTGGC CTTTGTCTCA CATGTTCTTT CCTGCGTTAT  
6961 CCCCTGATT TGTGGATAAC CGTATTACCG CCTTTGAGTG AGCTGATACC GTCGCGCGCA  
7021 GCCGAACGAC CGAGCGCAGC GAGTCAGTGA GCGAGGAAGC GGAAGAGCGC CCAATACGCA  
7081 AACCGCCTCT CCCCAGCGCT TGGCCGATT ATTAAATGCAG AGCTTGCAAT TCGCGCGTTT  
7141 TTCAATATTA TTGAAGCATT TATCAGGGTT ATTGTCTCAT GAGCGGATAC ATATTTGAAT  
7201 GTATTTAGAA AAATAAACAA ATAGGGGTTT CGCGCACATT TCCCCGAAAA GTGCCACCTG  
7261 ACGTCTAAGA AACCATTATT ATCATGACAT TAACCTATAA AAATAGGCGT AGTACGAGGC  
7321 CCTTCACTC ATTAGATGCA TGTCGTTACA TAACTTACGG TAAATGGCCC GCCTGGCTGA  
7381 CCGCCCAACG ACCCCCGCCC ATTGACGTCA ATAATGACGT ATGTTCCCAT AGTAACGCCA  
7441 ATAGGGACTT TCCATTGACG TCAATGGGTG GAGTATTTAC G

136/240  
FIGURE 47A

# pDEST 27 GST Amino Fusion in pCMV Sport-neo: Vector

CMV Promoter

600 // nac ggt ggg agg tct ata taa gca gag ctc gtt tag tga acc gtc aga tcy  
ntg cca ccc tcc aga tat att cgt ctc gag caa atc act tgg dag tct agc

651 cct gga gac gcc atc cac gct gtt ttg acc tcc ata gaa gac acc ggg acc  
gga cct ctg cgg tag gtg cga caa aac tgg agg tat ctt ctg tgg ccc tgg

702 gat cca gcc tcc gga ctc tag cct agg ccg cgg acc atg gcc cct ata ata  
cta ggt cgg agg cct gag atc gga tcc ggc gcc tgg tag cgg gga tat gat

753 ggt tat tgy aaa att aag ggc ctt gtg caa ccc act cga ctt ctt ttg gaa  
cca ata acc ttt taa ttc ccg gaa cac gtt ggg tga gct gaa gaa aac ctt

804 tat ctt gaa gaa aaa tat gaa gag cat ttg tat gag cgc gat gaa ggt gat  
ata gaa ctt ctt ttt ata ctt ctc gta aac ata ctc gcg cta ctt cca cta

1365 // ttt ggt ggt ggc gac cat cct cca aaa tcy gat ctg gtt ccg cgt tct aga  
aaa cca cca ccg ctg gta gga ggt ttt agc cta gac caa ggc gca aga tct

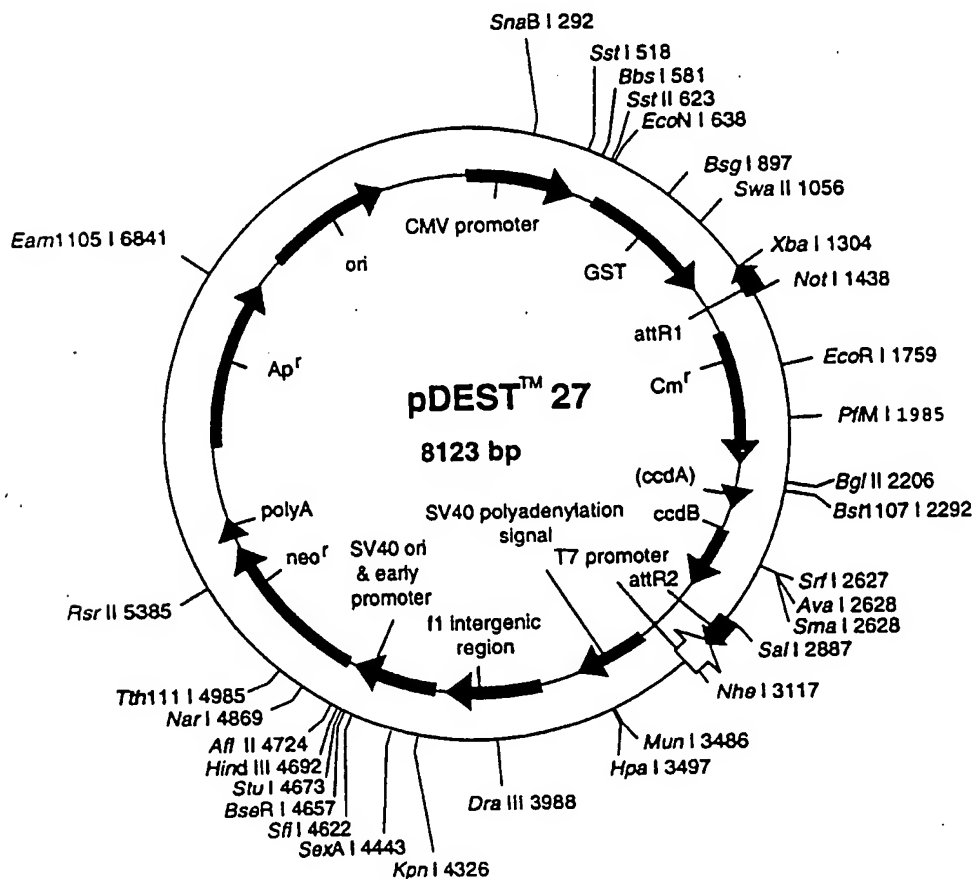
1416 tca aca agt ttg tac aaa aaa gct gaa cga gaa acg  
agt tgt tca aac atg ttg ttt cga ctt gct ctt tgc

Int attkl

Start Transl GST

M A P I L

V P R S R



137/240

## pDEST27 8123 bp (rotated to position 7800)

<u>Location (Base Nos.)</u>	<u>Gene Encoded</u>
130..793	GST
803..927	attR1
1036..1695	CmR
1815..1899	inactivated ccdA
2037..2342	ccdB
2383..2507	attR2
2693..3055	SV40 polyA
3250..3705	f1 intergenic region
3769..4187	SV40 promoter
4232..5026	neo
5090..5138	polyA
5549..6409	Apr
6558..7197	ori
7628..27	CMV promoter

```

1 ATAAGCAGAG CTCGTTTAGT GAACCGTCAG ATCGCCTGGA GACGCCATCC ACGCTGTTTT
61 GACCTCCATA GAAGACACCG GGACCGATCC AGCCTCCGGA CTCTAGCCTA GGCCGCGGAC
121 CATGGCCCTT ATACTAGGTT ATTGGAAAAT TAAGGGCCTT GTGCAACCCA CTCGACTTCT
181 TTTGGAATAT CTTGAAGAAA AATATGAAGA GCATTGTGAT GAGCGCGATG AAGGTGATAA
241 ATGGCGAAAC AAAAAGTTTG AATTGGGTTT GGAGTTTCCC AATCTTCCTT ATTATATTGA
301 TGGTGATGTT AAATTAACAC AGTCTATGGC CATCATACGT TATATAGCTG ACAAGCACAA
361 CATGTTGGGT GGTGTGCCAA AAGAGCGTGC AGAGATTTC AATGCTGAAG GAGCGGTTTT
421 GGATATTAGA TACGGTGTTC CGAGAATTGC ATATAGTAAA GACTTTGAAA CTCTCAAAAGT
481 TGATTTTCTT AGCAAGCTAC CTGAAATGCT GAAAATGTTC GAAGATCGTT TATGTCATAA
541 AACATATTTA AATGGTGATC ATGTAACCCA TCCTGACTTC ATGTTGTATG ACGCTCTTGA
601 TGTTGTTTTA TACATGGACC CAATGTGCCT GGATGCGTTC CCAAAATTAG TTTGTTTTAA
661 AAAACGTATT GAAGCTATCC CACAAATTGA TAAGTACTTG AAATCCAGCA AGTATATAGC
721 ATGGCCTTTG CAGGGCTGGC AAGCCACGTT TGGTGGTGGC GACCATCCTC CAAAATCGGA
781 TCTGGTCCG CGTTCTAGAT CAACAAGTTT GTACAAAAAA GCTGAACGAG AAACGTAAAA
841 TGATATAAAT ATCAATATAT TAAATTAGAT TTTGCATAAA AAACAGACTA CATAATACTG
901 TAAAACACAA CATATCCAGT CACTATGGCG GCCGCATTAG GCACCCAGG CTTTACACTT
961 TATGCTCCG GCTCGTATAA TGTGTGGATT TTGAGTTAGG ATCCGGCGAG ATTTTCAGGA
1021 GCTAAGGAAG CTAAATGGA GAAAAAATC ACTGGATATA CCACCGTTGA TATATCCCAA
1081 TGGCATCGTA AAGAACATTT TGAGGCATTT CAGTCAGTTG CTCAATGTAC CTATAACCAG
1141 ACCGTTTCTG TGATATTAC GGCCTTTTTA AAGACCGTAA AGAAAAATAA GCACAAGTTT
1201 TATCCGGCCT TTATTACAT TCTTGCCCGC CTGATGAATG CTCATCCGGA ATTCCGTATG
1261 GCAATGAAAG ACGGTGAGCT GGTGATATGG GATAGTGTTC ACCCTTGTTA CACCGTTTTT
1321 CATGAGCAAA CTGAAACGTT TTCATCGCTC TGGAGTGAAT ACCACGACGA TTTCCGGCAG
1381 TTTCTACACA TATATTCGCA AGATGTGGCG TGTTACGGTG AAAACCTGGC CTATTTCCCT
1441 AAAGGGTTTA TTGAGAATAT GTTTTTTCGT TCAGCCAATC CCTGGGTGAG TTTCACCACT
1501 TTTGATTTAA ACGTGGCCAA TATGGACAAC TTCTTCGCCC CCGTTTTCAC CATGGGCAAA
1561 TATTATACGC AAGGCGACAA GGTGCTGATG CCGCTGGCGA TTCAGGTTCA TCATGCCGTC
1621 TGTGATGGCT TCCATGTCGG CAGAATGCTT AATGAATTAC AACAGTACTG CGATGAGTGG
1681 CAGGGCGGGG CGTAAAGATC TGGATCCGGC TTAATAAAG CCAGATAACA GTATGCGTAT
1741 TTGCGCGCTG ATTTTTCGGG TATAAGAATA TATACTGATA TGTATACCCG AAGTATGTCA
1801 AAAAGAGGTG TGCTATGAAG CAGCGTATTA CAGTGACAGT TGACAGCGAC AGCTATCAGT
1861 TGCTCAAGGC ATATATGATG TCAATATCTC CGGTCTGGTA AGCACAACCA TGCAGAATGA
1921 AGCCCGTCGT CTGCGTGCCG AACGCTGGAA AGCGGAAAAT CAGGAAGGGA TGGCTGAGGT
1981 CGCCCGGTTT ATTGAAATGA ACGGCTCTTT TGCTGACGAG AACAGGGACT GGTGAAATGC
2041 AGTTTAAGGT TTACACCTAT AAAAGAGAGA GCCGTTATCG TCTGTTTGTG GATGTACAGA
2101 GTGATATTAT TGACACGCCC GGGCGACGGA TGGTGATCCC CCTGGCCAGT GCACGTCTGC
2161 TGTCAGATAA AGTCTCCCGT GAACTTTACC CGGTGGTGCA TATCGGGGAT GAAAGCTGGC
2221 GCATGATGAC CACCGATATG GCCAGTGTGC CGGTCTCCGT TATCGGGGAA GAAGTGGCTG
2281 ATCTCAGCCA CCGCGAAAAT GACATCAAAA ACGCCATTAA CCTGATGTTT TGGGGAATAT—

```

Figure 47B

138/240

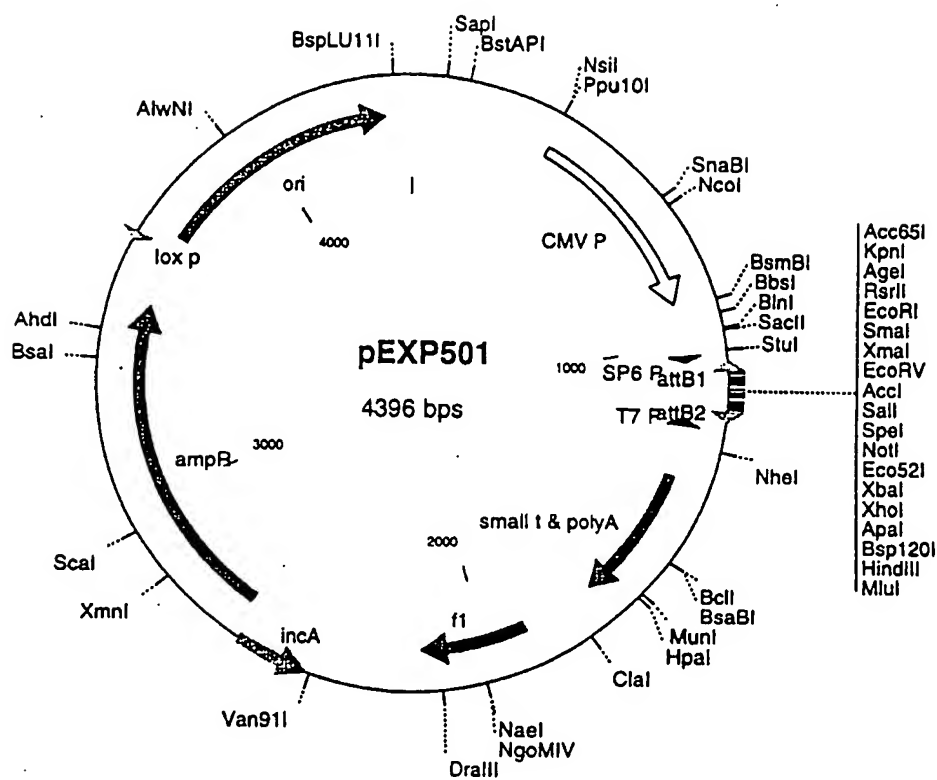
2341 AAATGTCAGG CTCCCTTATA CACAGCCAGT CTGCAGGTCG ACCATAGTGA CTGGATATGT  
2401 TGTGTTTTAC AGTATTATGT AGTCTGTTTT TTATGCAAAA TCTAATTTAA TATATTGATA  
2461 TTTATATCAT TTTACGTTTC TCGTTCAGCT TTCTTGTTACA AAGTGGTTGA TCGCGTGCAT  
2521 GCGACGTCAT AGCTCTCTCC CTATAGTGAG TCGTATTATA AGCTAGGCAC TGGCCGTCGT  
2581 TTTACAACGT CGTGACTGGG AAAACTGCTA GCTTGGGATC TTTGTGAAGG AACCTTACTT  
2641 CTGTGGTGTG ACATAATTGG ACAAACCTACC TACAGAGATT TAAAGCTCTA AGGTAATAT  
2701 AAAATTTTTA AGTGATAAT GTGTTAAACT AGCTGCATAT GCTTGCTGCT TGAGAGTTTT  
2761 GCTTACTGAG TATGATTTAT GAAAATATTA TACACAGGAG CTAGTGATTC TAATTGTTTG  
2821 TGTATTTTAG ATTACAGTC CCAAGGCTCA TTTCAGGCCC CTCAGTCCTC ACAGTCTGTT  
2881 CATGATCATA ATCAGCCATA CCACATTTGT AGAGGTTTTA CTTGCTTTAA AAAACCTCCC  
2941 ACACCTCCCC CTGAACCTGA AACATAAAAT GAATGCAATT GTTGTGTGTA ACTTGTTTAT  
3001 TGCAGCTTAT AATGGTTACA AATAAAGCAA TAGCATCACA AATTTCACAA ATAAAGCATT  
3061 TTTTTCCTG CATTCTAGTT GTGGTTTGTC CAAACTCATC AATGTATCTT ATCATGTCTG  
3121 GATCGATCCT GCATTAATGA ATCGGCCAAC GCGCGGGGAG AGGCGGTTTG CGTATTGGCT  
3181 GCGCTAATAG CGAAGAGGCC CGCACCGATC GCCCTTCCCA ACAGTTGCGC AGCCTGAATG  
3241 GCGAATGGGA CGCGCCCTGT AGCGGCGCAT TAAGCGCGGC GGGTGTGGTG GTTACGCGCA  
3301 GCGTGACCGC TACACTTGCC AGCGCCCTAG CGCCCGCTCC TTTCGCTTTC TTCCCTTCCT  
3361 TTCTCGCCAC GTTCGCCGCG TTTCCCGCTC AAGCTCTAAA TCGGGGGCTC CCTTTAGGGT  
3421 TCCGATTTAG TGCTTTACGG CACCTCGACC CCAAAAACT TGATTAGGGT GATGGTTCAC  
3481 GTAGTGGGCC ATCGCCCTGA TAGACGGTTT TTCGCCCTTT GACGTTGGAG TCCACGTCTT  
3541 TTAATAGTGG ACTCTGTTC CAAACTGGAA CAACACTCAA CCCTATCTCG GTCTATTCTT  
3601 TTGATTTATA AGGGATTTTG CCGATTTCCG CCTATTGGTT AAAAAATGAG CTGATTTAAC  
3661 AAATATTTAA CGCGAATTTT AACAAAATAT TAACGTTTAC AATTTCGCCT GATGCGGTAT  
3721 TTTCTCCTTA CGCATCTGTG CGGTATTTCA CACCGCATAC GCGGATCTGC GCAGCACCAT  
3781 GGCCTGAAAT AACCTCTGAA AGAGGAACTT GGTTAGGTAC CTTCTGAGGC GGAAGAACC  
3841 AGCTGTGGAA TGTGTGTCAG TTAGGGTGTG GAAAGTCCCC AGGCTCCCCA GCAGGCAGAA  
3901 GTATGCAAAG CATGCATCTC AATTAGTCAG CAACCAGGTG TGGAAAGTCC CCAGGCTCCC  
3961 CAGCAGGCAG AAGTATGCAA AGCATGCATC TCAATTAGTC AGCAACCATA GTCCCGCCCC  
4021 TAACTCCGCC CTAACCTCCG CCAGTTCGCG CCATTCTCCG CCTATTGGCT CCCCCTCCGA  
4081 GACTAATTTT TTTTATTTAT GCAGAGGCCG AGGCCGCCCTC GGCTCTGAG CTATTCCAGA  
4141 AGTAGTGAGG AGGCTTTTTT GGAGGCCTAG GCTTTTGCAA AAAGCTTGAT TCTTCTGACA  
4201 CAACAGTCTC GAACTTAAGG CTAGAGCCAC CATGATTGAA CAAGATGGAT TGCACGCAGG  
4261 TTCTCCGGCC GCTTGGGTGG AGAGGCTATT CGGCTATGAC TGGGCACAAC AGACAATCGG  
4321 CTGCTCTGAT GCCGCCGTGT TCCGGCTGTC AGCGCAGGGG CGCCCGGTTT TTTTGTCAA  
4381 GACCGACCTG TCCGGTGCCC TGAATGAAC TGCAGGACGAG GCAGCGCGGC TATCGTGGCT  
4441 GGCCACGACG GCGGTTCCCT GCGCAGCTGT GCTCGACGTT GTCAGTGAAG CGGGAAGGGA  
4501 CTGGCTGCTA TTGGGCGAAG TGCCGGGGCA GGATCTCTCG TCATCTCACC TTGCTCTGC  
4561 CGAGAAAGTA TCCATCATGG CTGATGCAAT GCGGCGGCTG CATACTGCTT ATCCGGCTAC  
4621 CTGCCCATTG GACCACCAAG CGAAACATCG CATCGAGCGA GCACGTACTC GGATGGAAGC  
4681 CGGTCTTGTC GATCAGGATG ATCTGGACGA AGAGCATCAG GGGCTCGCGC CAGCCGAACT  
4741 GTTCGCCAGG CTCAAGGCGC GCATGCCCCG CGGCGAGGAT CTCGTCGTGA CCCATGGCGA  
4801 TGCCTGCTTG CCGAATATCA TGGTGAAAA TGGCCGCTTT TCTGGATTCA TCGACTGTGG  
4861 CCGGCTGGGT GTGGCGGACC GCTATCAGGA CATAGCGTTG GCTACCCGTG ATATTGCTGA  
4921 AGAGCTTGCG GGCGAATGGG CTGACCGCTT CCTCGTGCTT TACGGTATCG CCGCTCCCGA  
4981 TTCGCAGCGC ATCGCCTTCT ATCGCCTTCT TGACGAGTTC TTCTGAGCGG GACTCTGGGG  
5041 TTCGAAATGA CCGACCAAGC GACGCCAAC CTGCCATCAC GATGGCCGCA ATAAAATATC  
5101 TTTATTTTCA TTACATCTGT GTGTTGGTTT TTTGTGTGAA TCGATAGCGA TAAGGATCCG  
5161 CGTATGGTGC ACTCTCAGTA CAATCTGCTC TGATGCCGCA TAGTTAAGCC AGCCCCGACA  
5221 CCCGCCAACA CCCGCTGACG CGCCCTGACG GGCTTGCTCG CTCCCGGCTC CCGCTTACAG  
5281 ACAAGCTGTG ACCGCTCCG GGAGCTGCAT GTGTCAGAGG TTTTCACCGT CATCAGCGAA  
5341 GCGCGCGAGA CGAAAGGGCC TCGTGATACG CCTATTTTGA TAGGTTAATG TCATGATAAT  
5401 AATGGTTTCT TAGACGTCAG GTGGCACTTT TCGGGGAAAT GTGCGCGGAA CCCCTATTTG  
5461 TTTATTTTTC TAAATACATT CAAATATGTA TCCGCTCATG AGACAATAAC CCTGATAAAT  
5521 GCTTCAATAA TATTGAAAAA GGAAGAGTAT GAGTATTCAA CATTCCGTG TCGCCCTTAT  
5581 TCCCTTTTTT GCGGCATTTT GCCTTCTCTG TTTTGCTCAC CCAGAAACGC TGGTGAAAGT  
5641 AAAAGATGCT GAAGATCAGT TGGGTGCACG AGTGGGTTAC ATCGAACTGG ATCTCAACAG  
5701 CCGTAAGATC CTTGAGAGTT TTCGCCCCGA AGAACGTTT CCAATGATGA GCACTTTAA  
5761 AGTTCGTGTA TGTGGCGCGG TATTATCCCG TATTGACGCC GGGCAAGAGC AACTCGGTCTG

FIGURE 47C



5821 CCGCATACAC TATTCTCAGA ATGACTTGGT TGAGTACTCA CCAGTCACAG AAAAGCATCT  
5881 TACGGATGGC ATGACAGTAA GAGAATTATG CAGTGCTGCC ATAACCATGA GTGATAACAC  
5941 TGCGGCCAAC TTACTTCTGA CAACGATCGG AGGACCGAAG GAGCTAACCG CTTTTTTGCA  
6001 CAACATGGGG GATCATGTAA CTCGCCTTGA TCGTTGGGAA CCGGAGCTGA ATGAAGCCAT  
6061 ACCAAACGAC GAGCGTGACA CCACGATGCC TGTAGCAATG GCAACAACGT TGCGCAAACT  
6121 ATTAACCTGGC GAACCTACTTA CTCTAGCTTC CCGGCAACAA TTAATAGACT GGATGGAGGC  
6181 GGATAAAGTT GCAGGACCAC TTCTGCGCTC GGCCCTTCCG GCTGGCTGGT TTATTGCTGA  
6241 TAAATCTGGA GCCGGTGAGC GTGGGTCTCG CGGTATCATT GCAGCACTGG GGCCAGATGG  
6301 TAAGCCCTCC CGTATCGTAG TTATCTACAC GACGGGGAGT CAGGCAACTA TGGATGAACG  
6361 AAATAGACAG ATCGCTGAGA TAGGTGCCCTC ACTGATTAAG CATTTGGTAACT TGTCAGACCA  
6421 AGTTTACTCA TATATACTTT AGATTGATTT AAAACTTCAT TTTTAATTTA AAAGGATCTA  
6481 GGTGAAGATC CTTTTTGATA ATCTCATGAC CAAAATCCCT TAACGTGAGT TTTCTGTCCA  
6541 CTGAGCGTCA GACCCCGTAG AAAAGATCAA AGGATCTTCT TGAGATCCTT TTTTCTCGG  
6601 CGTAATCTGC TGCTTGCAAA CAAAAAACC ACCGCTACCA GCGGTGGTTT GTTTGCCGGA  
6661 TCAAGAGCTA CCAACTCTTT TTCCGAAGGT AACTGGCTTC AGCAGAGCGC AGATAACCAA  
6721 TACTGTCTTT CTAGTGTAGC CGTAGTTAGG CCACCACTTC AAGAACTCTG TAGCACCGCC  
6781 TACATACCTC GCTCTGCTAA TCCTGTTACC AGTGCTGCT GCCAGTGGCG ATAAGTCGTG  
6841 TCTTACCGGG TTGGACTCAA GACGATAGTT ACCGGATAAG GCGCAGCGGT CGGGCTGAAC  
6901 GGGGGGTTTC TGCACACAGC CCAGCTTGGA GCGAACGACC TACACCGAAC TGAGATACCT  
6961 ACAGCGTGAG CATTGAGAAA GCGCCACGCT TCCCGAAGGG AGAAAGGCGG ACAGGTATCC  
7021 GGTAAGCGGC AGGGTCGGAA CAGGAGAGCG CACGAGGGAG CTTCCAGGGG GAAACGCCTG  
7081 GTATCTTTAT AGTCCTGTCT GGTTCGCCA CCTCTGACTT GAGCGTCGAT TTTTGTGATG  
7141 CTCGTCAGGG GGGCGGAGCC TATGGAAAA CGCCAGCAAC GCGGCCTTTT TACGGTTCCT  
7201 GGCCTTTTGC TGGCCTTTTG CTCACATGTT CTTTCTGCG TTATCCCCTG ATTCTGTGGA  
7261 TAACCGTATT ACCGCCTTTG AGTGAGCTGA TACCGCTCGC CGCAGCCGAA CGACCGAGCG  
7321 CAGCGAGTCA GTGAGCGAGG AAGCGGAAGA GCGCCCAATA CGCAAACCGC CTCTCCCCGC  
7381 GCGTTGGCCG ATTCAATTAAT GCAGAGCTTG CAATTCGCGC GTTTTTCAAT ATTATTGAAG  
7441 CATTTATCAG GGTATTGTCT TCATGAGCGG ATACATATTT GAATGTATTT AGAAAAATAA  
7501 ACAAATAGGG GTTCCGCGCA CATTTCCCCG AAAAGTGCCA CCTGACGTCT AAGAAACCAT  
7561 TATTATCATG ACATTAACCT ATAAAAATAG GCGTAGTACG AGGCCCTTTC ACTCATTAGA  
7621 TGCATGTCGT TACATAACTT ACGGTAATG GCCCGCTGG CTGACCGCCC AACGACCCCC  
7681 GCCCATTGAC GTCAATAATG ACGTATGTTT CCATAGTAAC GCCAATAGGG ACTTTCCATT  
7741 GACGTCAATG GGTGGAGTAT TTACGGTAAA CTGCCCCACTT GGCAGTACAT CAAGTGTATC  
7801 ATATGCCAAG TACGCCCCCT ATTGACGTCA ATGACGTAAT ATGGCCCGCC TGGCATTATG  
7861 CCCAGTACAT GACCTTATGG GACTTTCCTA CTTGGCAGTA CATCTACGTA TTAGTCATCG  
7921 CTATTACCAT GGTGATGCGG TTTTGGCAGT ACATCAATGG GCGTGGATAG CGGTTTGACT  
7981 CACGGGGATT TCCAAGTCTC CACCCCATG ACGTCAATGG GAGTTTGTGTT TGGCACCAAA  
8041 ATCAACGGGA CTTTCCAAAA TGTCGTAACA ACTCCGCCCC ATTGACGCAA ATGGGCGGTA  
8101 GCGTGTACG GTGGGAGGTC TAT

140/260

**Figure 4B A: pEXP501: pCMV.SPORT 6 host for attB Libraries**

141/240

**Figure 48B:** pEXP501 (cont'd). **Features of the att B cloning vector, pEXP501.** Bases within hatched area are replaced by cDNA in some LTI cDNA libraries.

868

---aga gct cgt tta gtg aac cgt cag atc gcc tgg aga cgc cat cca  
 ---tct cga gca aat cac ttg gca gtc tag cgg acc tct gcg gta ggt

CMV mRNA

cgc tgt ttt gac ctc cat aga aga cac cgg gac cga tcc agc ctc  
 gcg aca aaa ctg gag gta tct tct gtg gcc ctg gct agg tcc gag

Sst I

LTI rev primer

cgg act cta gcc tag gcc ggc gag cgg ata aca att tca cac agg  
 gcc tga gat cgg atc cgg cgc ctc gcc tat tgt taa agt gtg tcc

ABI rev primer

Stu

SP6 promoter

SP6

aaa cag cta tga cca tta ggc cta ttt agg tga cac tat aga aca  
 ttt gtc gat act ggt aat ccg gat aaa tcc act gtg ata tct tgt

Int

attB1

Age

Kpn

Rsr II

EcoRI

Sma

agt ttg tac aaa aaa gca ggc tgg tgc cgc tcc gga att ccc ggg  
 tca aac atg ttt ttt cgt ccg atg atg gcc agt cct taa ggg ccc

EcoRI

Sal

Spe

Not

Xba

atg tgc tgc agc agc tca tca gtc ggc ggc cgc tct aga gta tcc  
 tat agc agc tgc tgc agt gat gag ccg ccg gtc aga tct cat agg

Xho

Kpn

Kpn

Mlu

attB2

Int

dcc gag ggg cct agc ctt acg cgt acc cag ctt tct tgt aca aag  
 gag ctc dcc ggg ttc gaa tgc gca tgg gtc gaa aga aca tct ttc

agg dcc cta tag tga gtc gta tta taa gct agg cac tgg ccg tcc  
 acc agg gat atc act cag cat aat att cga tcc gtg acc ggc agc

T1

T1 promoter

ABI fwd

ttt tac aac gtc gtg act ggg aaa act cct agc ttg gga tct ttg---  
 aaa atg ttg cag cac tga ccc ttt tga cga tgg aac cct aga aac---

Nhe

1272

LTI fwd

142/240

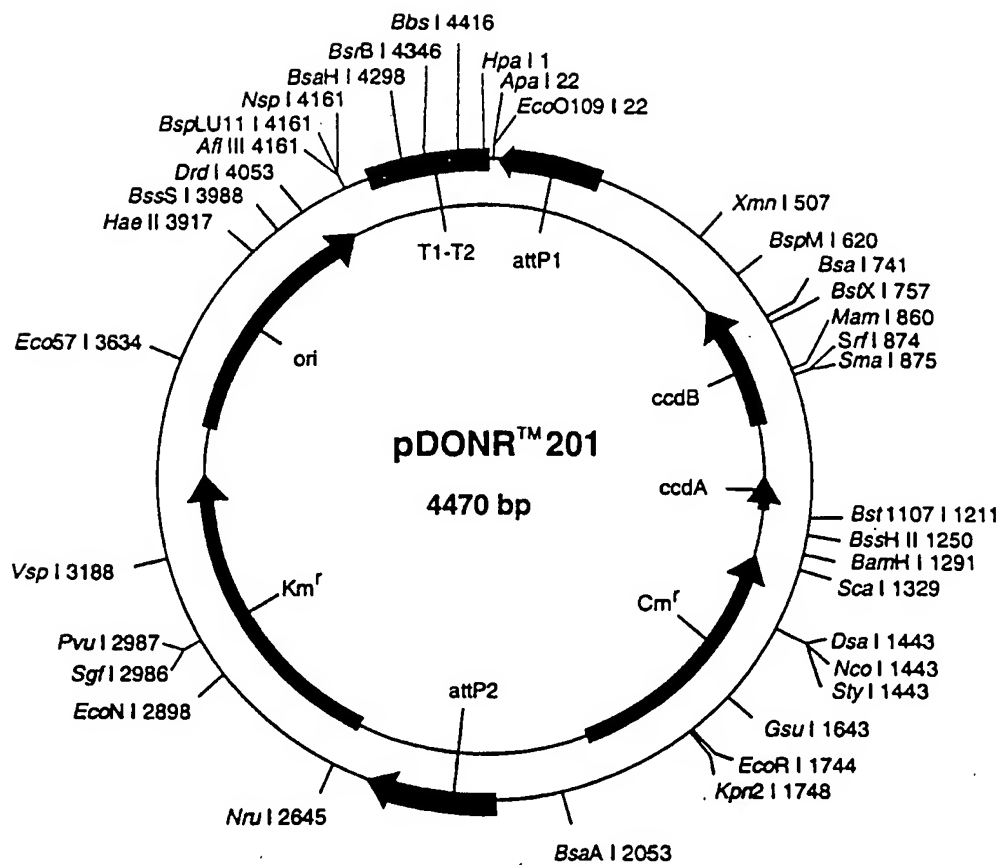
## pEXP501 4396 bp

```
1 CCATTGCGCCA TTCAGGCTGC GCAACTGTTG GGAAGGGCGA TCGGTGCGGG CCTCTTCGCT
61 ATTACGCCAG CCAATACGCA AACCGCCTCT CCCCGCGCGT TGGCCGATTG ATTAATGCAG
121 GATCGATCCA GACATGATAA GATACATTGA TGAGTTTGGA CAAACCACAA CTAGAATGCA
181 GTGAAAAAAA TGCTTTATTT GTGAAATTTG TGATGCTATT GCTTTATTTG TAACCATTAT
241 AAGCTGCAAT AAACAAGTTA ACAACAACAA TTGCATTGAT TTTATGTTTC AGGTTTCAGGG
301 GGAGGTGTGG GAGGTTTTTT AAAGCAAGTA AAACCTCTAC AAATGTGGTA TGGCTGATTA
361 TGATCATGAA CAGACTGTGA GGACTGAGGG GCCTGAAATG AGCCTTGGGA CTGTGAATCT
421 AAAATACACA AACAATTAGA ATCACTAGCT CCTGTGTATA ATATTTTCAT AAATCATACT
481 CAGTAAGCAA AACTCTCAAG CAGCAAGCAT ATGCAGCTAG TTTAACACAT TATACACTTA
541 AAAATTTTAT ATTTACCTTA GAGCTTTAAA TCTCTGTAGG TAGTTTGTC CATTATGTCA
601 CACCACAGAA GTAAGGTTCC TTCACAAAGA TCCCAAGCTA GCAGTTTTC CAGTCACGAC
661 GTTGTAAGAA GACGGCCAGT GCCTAGCTTA TAATACGACT CACTATAGGG AGCTTTTGT
721 ACAAGAAAGC TGGGTACGCG TAAGCTTGGG CCCCTCGAGG GATCCTCTAG AGCGGCCGCC
781 GACTAGTGAG CTCGTCGACG ATATCCCGGG AATTCCGGAC CGGTACCAGC CTGCTTTTTT
841 GTACAAACTT GTTCTATAGT GTCACCTAAA TAGGCCTAAT GGTTCATAGT GTTTCCTGTG
901 TGAAATTGTT ATCCGCTCCG CGGCCTAGGC TAGAGTCCGG AGGCTGGATC GGTCCTGGTG
961 TCTTCTATGG AGGTCAAAAC AGCGTGGATG GCGTCTCCAG GCGATCTGAC GGTTCATAAA
1021 ACGAGCTCTG CTTATATAGA CCTCCCACCG TACACGCCTA CCGCCCATTT CCGTCAATGG
1081 GCGGAGGTTG TTACGACATT TTGGAAGTC CCGTTGATTT TGGTGCCAAA ACAAACTCCC
1141 ATTGACGTCA ATGGGGTGGA GACTTGGAAA TCCCCGTGAG TCAAACCGCT ATCCACGCCC
1201 ATTGATGTAC TGCCAAAACC GCATCACCAT GGTAATAGCG ATGACTAATA CGTAGATGTA
1261 CTGCCAAGTA GGAAAGTCCC ATAAGGTCAT GTACTGGGCA TAATGCCAGG CGGGCCATTT
1321 ACCGTCATTG ACGTCAATAG GGGGCGTACT TGGCATATGA TACACTTGAT GTACTGCCAA
1381 GTGGGAGGTT TACCGTAAAT ACTCCACCCA TTGACGTCAA TGGAAAGTCC CTATTGGCGT
1441 TACTATGGGA ACATACGTCA TTATTGACGT CAATGGGCGG GGGTCGTGG GCGGTCAGCC
1501 AGGCGGGCCA TTTACCGTAA GTTATGTAAC GACATGCATC TAATGAGTGA AAGGGCCTCG
1561 TACTACGCCT ATTTTATAG GTTAATGTCA TGATAATAAT GGTTCCTTAG ACGTCAGGTG
1621 GCACGTTTCG GGGAAATGTG CGCGGAACCC CTATTTGTTT ATTTTCTAA ATACATTCAA
1681 ATATGTATCC GCTCATGAGA CAATAACCCT GATAAATGCT TCAATAATAT TGA AAAACGC
1741 GCGAATTGCA AGCTCTGCAT TAATGAATCG GCCAACGCGC GGGGAGAGGC GGTTCGCGTA
1801 TTGGGCGCTC TTCCGCTTCC TCGCTCACTG ACTCGCTGCG CTCGGTCGTT CGGCTGCGGC
1861 GAGCGGTATC AGCTCACTCA AAGGCGGTAA TACGGTTATC CACAGAATCA GGGGATAACG
1921 CAGGAAAGAA CATGTGAGCA AAAGGCCAGC AAAAGGCCAG GAACCGTAAA AAGGCCGCGT
1981 TGCTGGCGTT TTTCCATAGG CTCGCGCCCC CTGACGAGCA TCACAAAAAT GACGCTCAA
2041 GTGAGAGGTG GCGAAACCCG ACAGGACTAT AAAGATACCA GGCCTTTCCC CCTGGAAGCT
2101 CCCTCGTGCG CTCTCCTGTT CCGACCCTGC CGCTTACCGG ATACCTGTCC GCCTTTCTCC
2161 CTTGCGGAAG CGTGGCGCTT TCTCAATGCT CACGCTGTAG GTATCTCAGT TCGGTGTAGG
2221 TCGTTGCTC CAAGCTGGGC TGTGTGCACG AACCCCCCGT TCAGCCCGAC CGCTGCGCCT
2281 TATCCGGTAA CTATCGTCTT GAGTCCAACC CGGTAAAGCA CGACTTATCG CCACTGGCAG
2341 CAGCCACTGG TAACAGGATT AGCAGAGCGA GGTATGTAGG CGGTGCTACA GAGTTCTTGA
2401 AGTGGTGGCC TAACTACGGC TACACTAGAA GGACAGTATT TGGTATCTGC GCTCTGCTGA
2461 AGCCAGTTAC CTTCGGA AAAAGATTGGTA GCTCTTGATC CGGCAAACAA ACCACCGCTG
2521 GTAGCGGTGG TTTTTTTGTT TGCAAGCAGC AGATTACGCG CAGAAAAAAA GGATCTCAAG
2581 AAGATCCTTT GATCTTTTCT ACGGGGCTCTG ACGCTCAGTG GAACGAAAAC TCACGTTAAG
2641 GGATTTTGGT CATGCCATAA CTTCGTATAG CATACATTAT ACGAAGTTAT GGCATGAGAT
2701 TATCAAAAAG GATCTTCACC TAGATCCTTT TAAATTAAAA ATGAAGTTT AAATCAATCT
2761 AAAGTATATA TGAGTAAACT TGGTCTGACA GTTACCAATG CTTAATCAGT GAGGCACCTA
2821 TCTCAGCGAT CTGTCTATTT CGTTCATCCA TAGTTGCTTG ACTCCCCGTC GTGTAGATAA
2881 CTACGATACG GGAGGGCTTA CCATCTGGCC CCAGTGCTGC AATGATACCG CGAGACCCAC
2941 GCTCACCAGG TCCAGATTTA TCAGCAATAA ACCAGCCAGC CGGAAGGGCC GAGCGCAGAA
3001 GTGGTCCTGC AACTTTATCC GCCTCCATCC AGTCTATTAA TTGTTGCCGG GAAGCTAGAG
3061 TAAGTAGTTC GCCAGTTAAT AGTTTGCGCA ACGTTGTTGC CATTGCTACA GGCATCGTGG
3121 TGTCACGCTC GTCGTTTGGT ATGGCTTCAT TCAGCTCCGG TTCCCAACGA TCAAGGCGAG-
```

Figure 48C

3181 TTACATGATC CCCCATGTTG TGCAAAAAAG CGGTTAGCTC CTTCCGGTCCT CCGATCGTTG  
3241 TCAGAAGTAA GTTGGCCGCA GTGTTATCAC TCATGGTTAT GGCAGCACTG CATAATTCTC  
3301 TTA CTGTGCAT GCCATCCGTA AGATGCTTTT CTGTGACTGG TGAGTACTCA ACCAAGTCAT  
3361 TCTGAGAATA GTGTATGCGG CGACCGAGTT GCTCTTGCCC GGCCTCAATA CGGGATAATA  
3421 CCGCGCCACA TAGCAGAACT TTAAGAGTGC TCATCATTTG AAAACGTTCT TCGGGGCGAA  
3481 AACTCTCAAG GATCTTACCG CTGTTGAGAT CCAGTTCGAT GTAACCCACT CGTGCACCCA  
3541 ACTGATCTTC AGCATCTTTT ACTTTCACCA GCGTTTCTGG GTGAGCAAAA ACAGGAAGGC  
3601 AAAATGCCGC AAAAAAGGGA ATAAGGGCGA CACGGAATG TTGAATACTC ATACTCTTCC  
3661 TTTTCAATA TTATTGAAGC ATTTATCAGG GTTATTGTCT CATGCCAGGG GTGGGCACAC  
3721 ATATTTGATA CCAGCGATCC CTACACAGCA CATAATTCAA TGCGACTTCC CTCTATCGCA  
3781 CATCTTAGAC CTTTATTCTC CCTCCAGCAC ACATCGAAGC TGCCGAGCAA GCCGTTCTCA  
3841 CCAAGTCCAAG ACCTGGCATG AGCGGATACA TATTTGAATG TATTTAGAAA AATAAACAAA  
3901 TAGGGGTTCC GCGCACATTT CCCCAGAAAAG TGCCACCTGA AATTGTAAAC GTTAATATTT  
3961 TGTTAAAATT CGCGTTAAAT TTTTGTAAA TCAGCTCATT TTTTAACCAA TAGGCCGAAA  
4021 TCGGCAAAAT CCCTTATAAA TCAAAAAGAT AGACCGAGAT AGGGTTGAGT GTTGTTCAG  
4081 TTTGGAACAA GAGTCCACTA TTAAGAACG TGGACTCCAA CGTCAAAGGG CGAAAAACCG  
4141 TCTATCAGGG CGATGGCCCA CTACGTGAAC CATCACCTA ATCAAGTTTT TTGGGGTCGA  
4201 GGTGCCGTAA AGCACTAAAT CGGAACCCTA AAGGGAGCCC CCGATTAGA GCTTGACGGG  
4261 GAAAGCCGCG GAACGTGGCG AGAAAGGAAG GGAAGAAAGC GAAAGGAGCG GGCCTAGGG  
4321 CGCTGGCAAG TGTAGCGGTC ACCTGCGCG TAACCACCAC ACCCGCCGCG CTTAATGCGC  
4381 CGCTACAGGG CGCGTC

FIGURE 48D



145/240

## pDONR201 4470 bp (rotated to position 3516)

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
260..29		attP1
656..961		ccdB
1099..1184		ccdA
1303..1962		CmR
2210..2442		attP2
2565..3374		Kmr
3495..4134		ori

1	GTTAACGCTA	GCATGGATCT	CGGGCCCCAA	ATAATGATTT	TATTTTGACT	GATAGTGACC
61	TGTTTCGTTGC	AACAAATTGA	TGAGCAATGC	TTTTTTATAA	TGCCAACTTT	GTACAAAAAA
121	GCTGAACGAG	AAACGTAAAA	TGATATAAAT	ATCAATATAT	TAAATTAGAT	TTTGCATAAA
181	AAACAGACTA	CATAATACTG	TAAAACACAA	CATATCCAGT	CACTATGAAT	CAACTACTTA
241	GATGGTATTA	GTGACCTGTA	GTCGACCGAC	AGCCTTCCAA	ATGTTCTTCG	GGTGATGCTG
301	CCAACTTAGT	CGACCGACAG	CCTTCCAAAT	GTTCTTCTCA	AACGGAATCG	TCGTATCCAG
361	CCTACTCGCT	ATTGTCCTCA	ATGCCGTATT	AAATCATAAA	AAGAAATAAG	AAAAAGAGGT
421	GCGAGCCTCT	TTTTTGTGTG	ACAAAATAAA	AACATCTACC	TATTCATATA	CGTAGTGTC
481	ATAGTCCTGA	AAATCATCTG	CATCAAGAAC	AATTTACAA	CTCTTATACT	TTTCTCTTAC
541	AAGTCGTTCTG	GCTTCATCTG	GATTTTCAGC	CTCTATACTT	ACTAAACGTG	ATAAAGTTTC
601	TGTAATTTCT	ACTGTATCGA	CCTGCAGACT	GGCTGTGTAT	AAGGGAGCCT	GACATTTATA
661	TTCCCCAGAA	CATCAGGTTA	ATGGCGTTTT	TGATGTCATT	TTCGCGGTGG	CTGAGATCAG
721	CCACTTCTTC	CCCGATAACG	GAGACCGGCA	CACTGGCCAT	ATCGGTGGTC	ATCATGCGCC
781	AGCTTTTCATC	CCCGATATGC	ACCACCGGGT	AAAGTTCACG	GGAGACTTTA	TCTGACAGCA
841	GACGTGCACT	GGCCAGGGGG	ATCACCATCC	GTCGCCCGGG	CGTGTCATAA	ATATCACTCT
901	GTACATCCAC	AAACAGACGA	TAACGGCTCT	CTCTTTTATA	GGTGTAACC	TTAACTGCA
961	TTTCACCACT	CCCTGTTCTC	GTCAGCAAAA	GAGCCGTTCA	TTTCAATAAA	CCGGGCGACC
1021	TCAGCCATCC	CTTCCTGATT	TTCCGCTTTC	CAGCGTTCGG	CACGCAGACG	ACGGGCTTCA
1081	TTCTGCATGG	TTGTGCTTAC	CAGACCGGAG	ATATTGACAT	CATATATGCC	TTGAGCAACT
1141	GATAGCTGTC	GCTGTCAACT	GTCACGTGTA	TACGCTGCTT	CATAGCACAC	CTCTTTTGA
1201	CATACTTCGG	GTATACATAT	CAGTATATAT	TCTTATACCG	CAAAAATCAG	CGCGCAAATA
1261	CGCATACTGT	TATCTGGCTT	TTAGTAAGCC	GGATCCACGC	GATTACGCCC	CGCCCTGCCA
1321	CTCATCGCAG	TACTGTTGTA	ATTCAATTAAG	CATTCTGCCG	ACATGGAAGC	CATCACAGAC
1381	GGCATGATGA	ACCTGAATCG	CCAGCGGCAT	CAGCACCTTG	TCGCCTTGCG	TATAATATTT
1441	GCCCATGGTG	AAAACGGGGG	CGAAGAAGTT	GTCCATATTG	GCCACGTTTA	ATCAAAAAC
1501	GTTGAAACTC	ACCCAGGGAT	TGGCTGAGAC	GAAAAACATA	TTCTCAATAA	ACCTTTTAGG
1561	GAAATAGGCC	AGGTTTTCAC	CGTAACACGC	CACATCTTGC	GAATATATGT	GTAGAAACTG
1621	CCGGAATTCG	TCGTGGTATT	CACTCCAGAG	CGATGAAAC	GTTTCAGTTT	GCTCATGGAA
1681	AACGGTGTAA	CAAGGGTGAA	CACTATCCCA	TATCACCAGC	TCACCGTCTT	TCATTGCCAT
1741	ACGGAATTCC	GGATGAGCAT	TCATCAGGCG	GGCAAGAATG	TGAATAAAGG	CCGATAAAAA
1801	CTTGTGCTTA	TTTTTCTTTA	CGGTCTTTAA	AAAGGCCGTA	ATATCCAGCT	GAACGGTCTG
1861	GTTATAGGTA	CATTGAGCAA	CTGACTGAAA	TGCCCAAAA	TGTTCTTTAC	GATGCCATTG
1921	GGATATATCA	ACGGTGGTAT	ATCCAGTGAT	TTTTTCTCC	ATTTTAGCCT	CCTTAGCTCC
1981	TGAAAATCTC	GATAACTCAA	AAAATACGCC	CGGTAGTGAT	CTTATTTTCA	TATGGTGAAA
2041	GTTGGAACCT	CTTACGTGCC	GATCAACGTC	TCATTTTCGC	CAAAAGTTGG	CCCAGGGCTT
2101	CCCGGTATCA	ACAGGGACAC	CAGGATTTAT	TTATTCTGCG	AAGTGATCTT	CCGTACACAG
2161	TATTTATTCG	GCGCAAAGTG	CGTCGGGTGA	TGCTGCCAAC	TTAGTCGACT	ACAGGTCACT
2221	AATACCATCT	AAGTÀGTTGA	TTCATAGTGA	CTGGATATGT	TGTGTTTTAC	AGTATTATGT
2281	AGTCTGTTTT	TTATGCAAAA	TCTAATTTAA	TATATTGATA	TTATATTCAT	TTTACGTTTC
2341	TCGTTACAGT	TTCTTGTA	AAGTTGGCAT	TATAAGAAAG	CATTGCTTAT	CAATTTGTTG
2401	CAACGAACAG	GTCACATCA	GTCAAAATAA	AATCATTATT	TGCCATCCAG	CTGCAGCTCT
2461	GGCCCGTGTC	TCAAAATCTC	TGATGTTACA	TTGCACAAGA	TAAAAATATA	TCATCATGAA
2521	CAATAAACT	GTCTGCTTAC	ATAAACAGTA	ATACAAGGGG	TGTTATGAGC	CATATTCAAC
2581	GGGAAACGTC	GAGGCCGCGA	TTAAATTCCA	ACATGGATGC	TGATTTATAT	GGGTATAAAT
2641	GGGCTCGCGA	TAATGTCGGG	CAATCAGGTG	CGACAATCTA	TCGCTTGAT	GGGAAGCCCG
2701	ATGCGCCAGA	GTTGTTTCTG	AAACATGGCA	AAGGTAGCGT	TGCCAATGAT	GTTACAGATG ~

FIGURE 49B

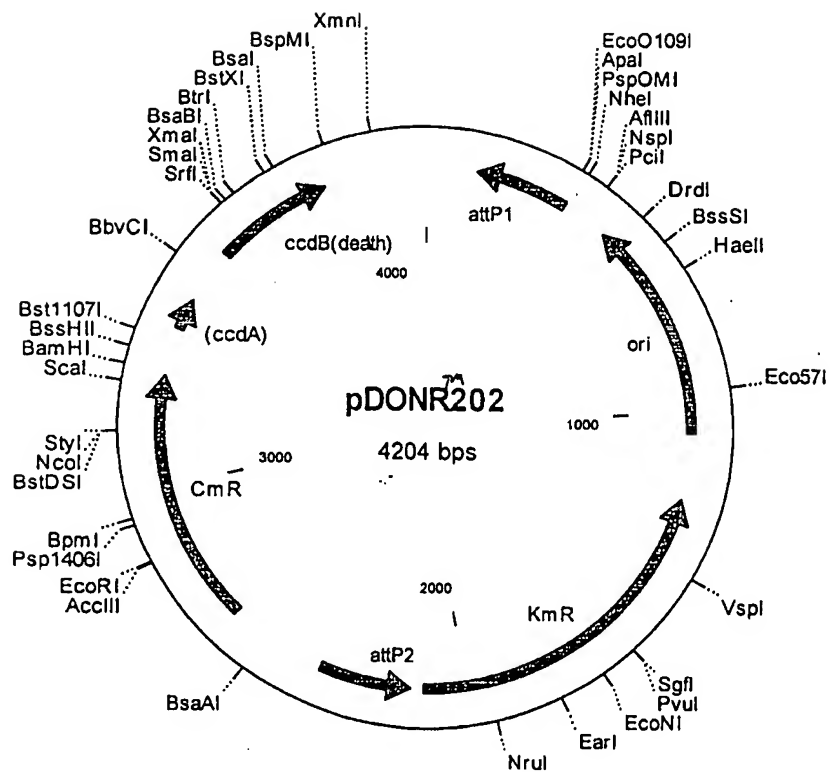
146/240

2761 AGATGGTCAG ACTAAACTGG CTGACGGAAT TTATGCCTCT TCCGACCATC AAGCATTTTA  
2821 TCCGTACTCC TGATGATGCA TGGTTACTCA CCACTGCGAT CCCC GGAAAA ACAGCATTCC  
2881 AGGTATTAGA AGAATATCCT GATTCAGGTG AAAATATTGT TGATGCGCTG GCAGTGTTCC  
2941 TCGCGCCGTT GCATTTCGATT CCTGTTTGTA ATTGTCCTTT TAACAGCGAT CGCGTATTTT  
3001 GTCTCGCTCA GCGCGCAATCA CGAATGAATA ACGGTTTGGT TGATGCGAGT GATTTTGATG  
3061 ACGAGCGTAA TGGCTGGCCT GTTGAACAAG TCTGGAAAAGA AATGCATAAA CTTTTGCCAT  
3121 TCTCACC GGA TTCAGTCGTC ACTCATGGTG ATTTCTCACT TGATAACCTT ATTTTGTACG  
3181 AGGGGAAATT AATAGGTTGT ATTGATGTTG GACGAGTCGG AATCGCAGAC CGATACCAGG  
3241 ATCTTGCCAT CCTATGGAAC TGCCTCGGTG AGTTTTCTCC TTCATTACAG AAACGGCTTT  
3301 TTCAAAAATA TGGTATTGAT AATCCTGATA TGAATAAATT GCAGTTTCAT TTGATGCTCG  
3361 ATGAGTTTTT CTAATCAGAA TTGGTTAATT GGTGTGAACA CTGGCAGAGC ATTACGCTGA  
3421 CTTGACGGGA CGGCGCAAGC TCATGACCAA AATCCCTTAA CGTGAGTTTT CGTTCCACTG  
3481 AGCGTCAGAC CCCGTAGAAA AGATCAAAGG ATCTTCTTGA GATCCTTTTT TTCTGCGCGT  
3541 AATCTGCTGC TTGCAACAAA AAAAACCACC GCTACCAGCG GTGGTTTGTT TGCCGGATCA  
3601 AGAGCTACCA ACTCTTTTTC CGAAGGTAAC TGGCTTCAGC AGAGCGCAGA TACCAAATAC  
3661 TGTCTTCTA GTGTAGCCGT AGTTAGGCCA CCACTTCAAG AACTCTGTAG CACCGCCTAC  
3721 ATACCTCGCT CTGCTAATCC TGTTACCAGT GGCTGCTGCC AGTGGCGATA AGTCGTGTCT  
3781 TACCGGGTTG GACTCAAGAC GATAGTTACC GGATAAGGCG CAGCGGTCGG GCTGAACGGG  
3841 GGGTTCGTGC ACACAGCCCA GCTTGAGAGC AACGACCTAC ACCGAACTGA GATACCTACA  
3901 GCGTGAGCTA TGAGAAAGCG CCACGCTTCC CGAAGGGAGA AAGGCGGACA GGTATCCGGT  
3961 AAGCGGCAGG GTCGGAACAG GAGAGCGCAC GAGGGAGCTT CCAGGGGGAA ACGCCTGGTA  
4021 TCTTTATAGT CCTGTCGGGT TTCGCCACCT CTGACTTGAG CGTCGATTTT TGTGATGCTC  
4081 GTCAGGGGGG CGGAGCCTAT GGA AAAACGC CAGCAACGCG GCCTTTTTAC GGTTCCTGGC  
4141 CTTTTGCTGG CCTTTTGCTC ACATGTTCTT TCCTGCGTTA TCCCCTGATT CTGTGGATAA  
4201 CCGTATTACC GCTAGCCAGG AAGAGTTTGT AGAAACGCAA AAAGGCCATC CGTCAGGATG  
4261 GCCTTCTGCT TAGTTTGATG CCTGGCAGTT TATGGCGGGC GTCCTGCCCG CCACCCTCCG  
4321 GGCCGTTGCT TCACAACGTT CAAATCCGCT CCCGGCGGAT TTGTCCTACT CAGGAGAGCG  
4381 TTCACCGACA AACAACAGAT AAAACGAAAG GCCCAGTCTT CCGACTGAGC CTTTCGTTTT  
4441 ATTTGATGCC TGGCAGTTCC CTACTCTCGC

FIGURE 49C



147/240  
FIGURE 50A: pDONR202 (kan<sup>R</sup>)



148/240

## pDONR202 4204 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
369..127		attP1
486..1059		ori
1228..2107		KmR
2381..2140		attP2
2629..3288		CmR
3408..3492		inactivated ccdA
3630..3935		ccdB

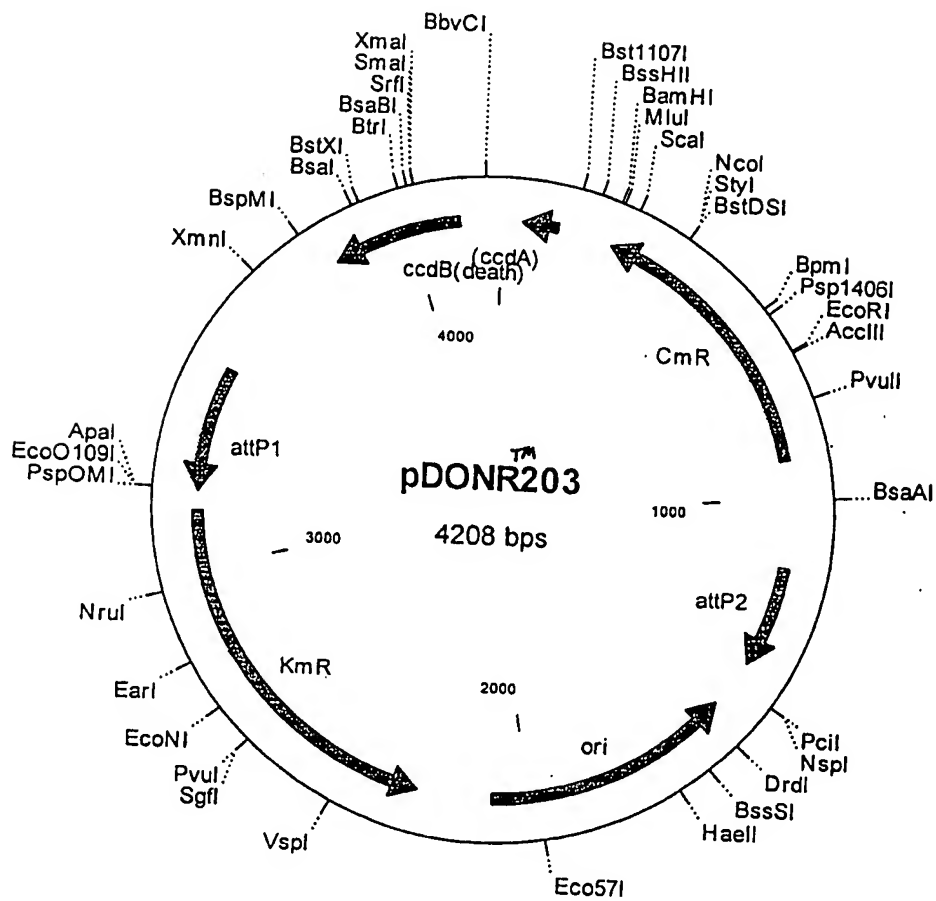
1	CGGCATTGAG	GACAATAGCG	AGTAGGCTGG	ATACGACGAT	TCCGTTTGAG	AAGAACATTT
61	GGAAGGCTGT	CGGTCGACTA	AGTTGGCAGC	ATCACCCGAA	GAACATTTGG	AAGGCTGTCTG
121	GTCGACTACA	GGTCACTAAT	ACCATCTAAG	TAGTTGATTC	ATAGTGACTG	GATATGTTGT
181	GTTTTACAGT	ATTATGTAGT	CTGTTTTTTA	TGCAAAATCT	AATTTAATAT	ATTGATATTT
241	ATATCATTTT	ACGTTTCTCG	TTCAGCTTTT	TTGTACAAAG	TTGGCATTAT	AAAAAAGCAT
301	TGCTCATCAA	TTTGTGCAA	CGAACAGGTC	ACTATCAGTC	AAAATAAAAT	CATTATTGCG
361	GGCCCGAGAT	CCATGCTAGC	GGTAATACGG	TTATCCACAG	AATCAGGGGA	TAACGCAGGA
421	AAGAACATGT	GAGCAAAAGG	CCAGCAAAAG	GCCAGGAACC	GTAAAAAGGC	CGCGTTGCTG
481	CGGTTTTTCC	ATAGGCTCCG	CCCCCTGAC	GAGCATCACA	AAAATCGACG	CTCAAGTCAG
541	AGGTGGCGAA	ACCCGACAGG	ACTATAAAGA	TACCAGGCGT	TTCCCCCTGG	AAGCTCCCTC
601	GTGCGCTCTC	CTGTTCCGAC	CCTGCCGCTT	ACCGGATACC	TGTCCGCTT	TCTCCCTTCG
661	GGAAGCGTGG	CGCTTTCTCA	TAGCTCACGC	TGTAGGTATC	TCAGTTCGGT	GTAGGTCGTT
721	CGTCTCAAGC	TGGGCTGTGT	GCACGAACCC	CCCGTTCAGC	CCGACCGCTG	CGCCTTATCC
781	GGTAACATATC	GTCTTGAGTC	CAACCCGTA	AGACACGACT	TATCGCCACT	GGCAGCAGCC
841	ACTGGTAACA	GGATTAGCAG	AGCGAGGTAT	GTAGGCGGTG	CTACAGAGTT	CTTGAAGTGG
901	TGGCCTAACT	ACGGCTACAC	TAGAAGGACA	GTATTGGTA	TCTGCGCTCT	GCTGAAGCCA
961	GTTACCTTCG	GAAAAAGAGT	TGGTAGCTCT	TGATCCGGCA	AACAAACCAC	CGCTGGTAGC
1021	GGTGGTTTTT	TTGTTTGCAA	GCAGCAGATT	ACGCGCAGAA	AAAAAGGATC	TCAAGAAGAT
1081	CCTTTGATCT	TTTCTACGGG	GTCTGACGCT	CAGTGAACG	AAAACCTCAG	TTAAGGGATT
1141	TTGGTCATGA	GCTTGCGCCG	TCCCGTCAAG	TCAGCGTAAT	GCTCTGCCAG	TGTTACAACC
1201	AATTAAACCA	TTCTGATTAG	AAAAACTCAT	CGAGCATCAA	ATGAAACTGC	AATTTATTCA
1261	TATCAGGATT	ATCAATACCA	TATTTTTGAA	AAAGCCGTTT	CTGTAATGAA	GGAGAAAACT
1321	CACCGAGGCA	GTTCCATAGG	ATGGCAAGAT	CCTGGTATCG	GTCTGCGATT	CCGACTCGTC
1381	CAACATCAAT	ACAACCTATT	AATTTCCCTT	CGTCAAAAAT	AAGGTTATCA	AGTGAGAAAT
1441	CACCATGAGT	GACGACTGAA	TCCGGTGAGA	ATGGCAAAAG	TTTATGCATT	TCTTTCCAGA
1501	CTTGTTCAAC	AGGCCAGCCA	TTACGCTCGT	CATCAAAATC	ACTCGCATCA	ACCAAACCGT
1561	TATTCATTCG	TGATTGCGCC	TGAGCGAGAC	GAAATACGCG	ATCGCTGTTA	AAAGGACAAT
1621	TACAAACAGG	AATCGAATGC	AACCGGCGCA	GGAACACTGC	CAGCGCATCA	ACAAATTTTT
1681	CACCTGAATC	AGGATATTCT	TCTAATACCT	GGAATGCTGT	TTTTCCGGGG	ATCGCAGTGG
1741	TGAGTAACCA	TGCATCATCA	GGAGTACGGA	TAAAATGCTT	GATGGTCGGA	AGAGGCATAA
1801	ATTCCGTCAG	CCAGTTTAGT	CTGACCATCT	CATCTGTAAC	ATCATTGGCA	ACGCTACCTT
1861	TGCCATGTTT	CAGAAACAAC	TCTGGCGCAT	CGGGCTTCCC	ATACAAGCGA	TAGATTGTCTG
1921	CACCTGATTG	CCCAGACATTA	TCGCGAGCCC	ATTTATACCC	ATATAAATCA	GCATCCATGT
1981	TGGAATTTAA	TCGCGGCCCTC	GACGTTTCCC	GTTGAATATG	GCTCATAACA	CCCCTTGTAT
2041	TACTGTTTAT	GTAAGCAGAC	AGTTTTTATTG	TTTATGATGA	TATATTTTTA	TCTTGTGCAA
2101	TGTAACATCA	GAGATTTTGA	GACACGGGCC	AGAGCTGCAG	CTGGATGGCA	AATAATGATT
2161	TTATTTTGAC	TGATAGTGAC	CTGTTCTGTTG	CAACAAATTG	ATAAGCAATG	CTTTCTTATA
2221	ATGCCAACTT	TGTACAAGAA	AGCTGAACGA	GAAACGTAAA	ATGATATAAA	TATCAATATA
2281	TTAAATTAGA	TTTTGCATAA	AAAACAGACT	ACATAATACT	GTAAAACACA	ACATATCCAG
2341	TCACTATGAA	TCAACTACTT	AGATGGTATT	AGTGACCTGT	AGTCGACTAA	GTTGGCAGCA
2401	TCACCCGACG	CACCTTTCGC	CGAATAAATA	CCTGTGACGG	AAGATCACTT	CGCAGAATAA
2461	ATAAATCCTG	GTGTCCCTGT	TGATACCGGG	AAGCCCTGGG	CCAACCTTTG	GCGAAAATGA
2521	GACGTTGATC	GGCACGTAAG	AGGTTCCAAC	TTTCACCATA	ATGAAATAAG	ATCACTACCG
2581	GGCGTATTTT	TTGAGTTATC	GAGATTTTCA	GGAGCTAAGG	AAGCTAAAAT	GGAGAAAAAA
2641	ATCACTGGAT	ATACCACCGT	TGATATATCC	CAATGGCATC	GTAAAGAACA	TTTTGAGGCA
2701	TTTCAGTCAG	TTGCTCAATG	TACCTATAAC	CAGACCGTTC	AGCTGGATAT	TACGGCCTTT -

FIGURE 50B

149/240

2761 TTAAAGACCG TAAAGAAAA TAAGCACAAG TTTTATCCGG CCTTTATTCA CATTCTTGCC  
2821 CGCCTGATGA ATGCTCATCC GGAATTCCGT ATGGCAATGA AAGACGGTGA GCTGGTGATA  
2881 TGGGATAGTG TTCACCCCTG TTACACCGTT TTCCATGAGC AAAC TGAAAC GTTTTCATCG  
2941 CTCTGGAGTG AATACCACGA CGATTTCCGG CAGTTTCTAC ACATATATTC GCAAGATGTG  
3001 GCGTGTTACG GTGAAAACCT GGCCTATTTT CCTAAAGGGT TTATTGAGAA TATGTTTTTC  
3061 GTCTCAGCCA ATCCCTGGGT GAGTTTCACC AGTTTTGATT TAAACGTGGC CAATATGGAC  
3121 AACTTCTTCG CCCCCGTTTT CACCATGGGC AAATATTATA CGCAAGGCGA CAAGGTGCTG  
3181 ATGCCGCTGG CGATTACAGT TCATCATGCC GTCTGTGATG GCTTCCATGT CGGCAGAATG  
3241 CTTAATGAAT TACAACAGTA CTGCGATGAG TGGCAGGGCG GGGCGTAATC GCGTGGATCC  
3301 GGCTTACTAA AAGCCAGATA ACAGTATGCG TATTTGCGCG CTGATTTTTG CCGTATAAGA  
3361 ATATATACTG ATATGTATAC CCGAAGTATG TCAAAAAGAG GTGTGCTATG AAGCAGCGTA  
3421 TTACAGTGAC AGTTGACAGC GACAGCTATC AGTTGCTCAA GGCATATATG ATGTCAATAT  
3481 CTCCGGTCTG GTAAGCACAA CCATGCAGAA TGAAGCCCGT CGTCTGCGTG CCGAACGCTG  
3541 GAAAGCGGAA AATCAGGAAG GGATGGCTGA GGTCGCCCCG TTTATTGAAA TGAACGGCTC  
3601 TTTTGCTGAC GAGAACAGGG ACTGGTGAAA TGCAGTTTAA GGTTTACACC TATAAAAGAG  
3661 AGAGCCGTTA TCGTCTGTTT GTGGATGTAC AGAGTGATAT TATTGACACG CCCGGGCGAC  
3721 GGATGGTGAT CCCCCTGGCC AGTGCACGTC TGCTGTCAGA TAAAGTCTCC CGTGAACTTT  
3781 ACCCGGTGGT GCATATCGGG GATGAAAGCT GGCGCATGAT GACCACCGAT ATGGCCAGTG  
3841 TGCCGGTCTC CGTTATCGGG GAAGAAAGTG CTGATCTCAG CCACCGCGAA AATGACATCA  
3901 AAAACGCCAT TAACCTGATG TTCTGGGGAA TATAAATGTC AGGCTCCCTT ATACACAGCC  
3961 AGTCTGCAGG TCGATACAGT AGAAATTACA GAAACTTTAT CACGTTTAGT AAGTATAGAG  
4021 GCTGAAAATC CAGATGAAGC CGAACGACTT GTAAGAGAAA AGTATAAGAG TTGTGAAATT  
4081 GTTCTTGATG CAGATGATTT TCAGGACTAT GACACTAGCG TATATGAATA GGTAGATGTT  
4141 TTTATTTTGT CACACAAAA AGAGGCTCGC ACCTCTTTTT CTTATTTCTT TTTATGATTT  
4201 AATA

FIGURE 50C

FIGURE 5/A pDONR203 (kan<sup>R</sup>)

151/240

## pDONR203 4208 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
47..131		inactivated ccdA
251..910		CmR
1158..1398		attP2
1509..2082		ori
2251..3130		KmR
3464..3174		attP1
3812..4117		ccdB

1	GCGTTCGGCA	CGCAGACGAC	GGGCTTCATT	CTGCATGGTT	GTGCTTACCA	GACCGGAGAT
61	ATTGACATCA	TATATGCCTT	GAGCAACTGA	TAGCTGTGCG	TGTCAACTGT	CACTGTAATA
121	CGCTGCTTCA	TAGCACACCT	CTTTTTGACA	TACTTCGGGT	ATACATATCA	GTATATATTC
181	TTATACCGCA	AAAATCAGCG	CGCAAATACG	CATACTGTTA	TCTGGCTTTT	AGTAAGCCGG
241	ATCCACGCGT	TTACGCCCCG	CCCTGCCACT	CATCGCAGTA	CTGTTGTAAT	TCATTAAGCA
301	TTCTGCCGAC	ATGGAAGCCA	TCACAGACGG	CATGATGAAC	CTGAATCGCC	AGCGGCATCA
361	GCACCTTGTC	GCCTTGCGTA	TAATATTTGC	CCATGGTGAA	AACGGGGGCG	AAGAAGTTGT
421	CCATATTGGC	CACGTTTAAA	TCAAACTGG	TGAACTCAC	CCAGGGATTG	GCTGAGACGA
481	AAAACATATT	CTCAATAAAC	CCTTTAGGGA	AATAGGCCAG	GTTTTACCG	TAACACGCCA
541	CATCTTGCGA	ATATATGTGT	AGAAACTGCC	GGAAATCGTC	GTGGTATTCA	CTCCAGAGCG
601	ATGAAAACGT	TTCAGTTTGC	TCATGGAAAA	CGGTGTAACA	AGGGTGAACA	CTATCCCAT
661	TCACCAGCTC	ACCGTCTTTC	ATTGCCATAC	GGAATTCCGG	ATGAGCATT	ATCAGGCGGG
721	CAAGAATGTG	AATAAAGGCC	GGATAAAACT	TGTGCTTATT	TTTCTTTACG	GTCTTTAAAA
781	AGGCCGTAAT	ATCCAGCTGA	ACGGTCTGGT	TATAGGTACA	TTGAGCAACT	GACTGAAATG
841	CCTCAAAATG	TTCTTTACGA	TGCCATTGGG	ATATATCAAC	GGTGGTATAT	CCAGTGATTT
901	TTTTCTCCAT	TTTAGCTTCC	TTAGCTCCTG	AAAATCTCGA	TAACCAAAA	AAATACGCCCG
961	GTAGTGATCT	TATTTTCATTA	TGGTGAAAGT	TGGAACCTCT	TACGTGCCGA	TCAACGTCTC
1021	ATTTTCGCCA	AAAGTTGGCC	CAGGGCTTCC	CGGTATCAAC	AGGGACACCA	GGATTTATTT
1081	ATTCTGCGAA	GTGATCTTCC	GTACACAGTA	TTTATTCGGC	GCAAAGTGCG	TCGGGTGATG
1141	CTGCCAACTT	AGTCGACTAC	AGGTCATAA	TACCATCTAA	GTAGTTGATT	CATAGTGACT
1201	GGATATGTTG	TGTTTTACAG	TATTATGTAG	TCTGTTTTTT	ATGCAAAATC	TAATTTAATA
1261	TATTGATATT	TATATCATTT	TACGTTTCTC	GTTCAGCTTT	CTTGTACAAA	GTTGGCATT
1321	TAAGAAAGCA	TTGCTTATCA	ATTTGTTGCA	ACGAACAGGT	CACTATCAGT	CAAAATAAAA
1381	TCATTATTTG	CCATCCAGCT	AGCGGTAATA	CGGTTATCCA	CAGAATCAGG	GGATAACGCA
1441	GGAAAGAACA	TGTGAGCAAA	AGGCCAGCAA	AAGGCCAGGA	ACCGTAAAAA	GGCCGCGTTG
1501	CTGGCGTTTT	TCCATAGGCT	CCGCCCCCTT	GACGAGCATC	ACAAAAATCG	ACGCTCAAGT
1561	CAGAGGTGGC	GAAACCCGAC	AGGACTATAA	AGATAACAGG	CGTTTTCCCC	TGGAAGCTCC
1621	CTCGTGCGCT	CTCCTGTTCC	GACCCGCGCG	CTTACCGGAT	ACCTGTCCGC	CTTTCTCCCT
1681	TCGGGAAGCG	TGGCGCTTTC	TCATAGCTCA	CGCTGTAGGT	ATCTCAGTTC	GGTGTAGGTC
1741	GTTTCGCTCCA	AGCTGGGCTG	TGTGCACGAA	CCCCCGTTTC	AGCCCGACCG	CTGCGCCTTA
1801	TCCGGTAACT	ATCGTCTTGA	GTCCAACCCG	GTAAGACACG	ACTTATCGCC	ACTGGCAGCA
1861	GCCACTGGTA	ACAGGATTAG	CAGAGCGAGG	TATGTAGGCG	GTGCTACAGA	GTTCTTGAAG
1921	TGGTGGCCTA	ACTACGGCTA	CACTAGAAGA	ACAGTATTTG	GTATCTGCGC	TCTGCTGAAG
1981	CCAGTTACCT	TCGGAAAAAG	AGTTGGTAGC	TCTTGATCCG	GCAAAACAAAC	CACCGCTGGT
2041	AGCGGTGGTT	TTTTTGTTTG	CAAGCAGCAG	ATTACGCGCA	GAAAAAAGG	ATCTCAAGAA
2101	GATCCTTTGA	TCTTTTCTAC	GGGGTCTGAC	GCTCAGTGGA	ACGAAAACTC	ACGTTAAGGG
2161	ATTTTGGTCA	TGAGCTTGCG	CCGTCCCGTC	AAGTCAGCGT	AATGCTCTGC	CAGTGTTACA
2221	ACCAATTAAC	CAATTCTGAT	TAGAAAAACT	CATCGAGCAT	CAAATGAAAC	TGCAATTTAT
2281	TCATATCAGG	ATTATCAATA	CCATATTTTT	GAAAAAGCCG	TTTCTGTAAT	GAAGGAGAAA
2341	ACTCACCGAG	GCAGTTCCAT	AGGATGGCAA	GATCCTGGTA	TCGGTCTGCG	ATTCCGACTC
2401	GTCCAACATC	AATACAACCT	ATTAATTTC	CCTCGTCAA	AATAAGGTTA	TCAAGTGAGA
2461	AATCACCATG	AGTGACGACT	GAATCCGGTG	AGAATGGCAA	AAGTTTATGC	ATTTCTTTCC
2521	AGACTTGTTT	AACAGGCCAG	CCATTACGCT	CGTCATCAAA	ATCACTCGCA	TCAACCAAAC
2581	CGTTATTTCAT	TCGTGATTGC	GCCTGAGCGA	GACGAAATAC	GCGATCGCTG	TTAAAAGGAC
2641	AATTACAAAC	AGGAATCGAA	TGCAACCGGC	GCAGGAACAC	TGCCAGCGCA	TCAACAATAT
2701	TTTCACCTGA	ATCAGGATAT	TCTTCTAATA	CCTGGAATGC	TGTTTTTCCG	GGGATCGCAG-

Figure 51B

2761 TGGTGAGTAA CCATGCATCA TCAGGAGTAC GGATAAAATG CTTGATGGTC GGAAGAGGCA  
2821 TAAATTCCGT CAGCCAGTTT AGTCTGACCA TCTCATCTGT AACATCATTG GCAACGCTAC  
2881 CTTTGCCATG TTTCAGAAAC AACTCTGGCG CATCGGGCTT CCCATACAAG CGATAGATTG  
2941 TCGCACCTGA TTGCCCCGACA TTATCGCGAG CCCATTTATA CCCATATAAA TCAGCATCCA  
3001 TGTTGGAATT TAATCGCGGC CTCGACGTTT CCCGTTGAAT ATGGCTCATA ACACCCCTTG  
3061 TATTACTGTT TATGTAAGCA GACAGTTTAA TTGTTTCATGA TGATATATTT TTATCTTGTG  
3121 CAATGTAACA TCAGAGATTT TGAGACACGG GCCAGAGCTG CAGCTAGCAT GGATCTCGGG  
3181 CCCCAAATAA TGATTTTATT TTGACTGATA GTGACCTGTT CGTTGCAACA AATTGATGAG  
3241 CAATGCTTTT TTATAATGCC AACTTTGTAC AAAAAAGCTG AACGAGAAAC GTAAATGAT  
3301 ATAAATATCA ATATATTAAA TTAGATTTTG CATAAAAAAC AGACTACATA ATACTGTAAA  
3361 ACACAACATA TCCAGTCACT ATGAATCAAC TACTTAGATG GTATTAGTGA CCTGTAGTCG  
3421 ACCGACAGCC TTCCAAATGT TCTTCGGGTG ATGCTGCCAA CTTAGTCGAC CGACAGCCTT  
3481 CCAAATGTTT TTCTCAAACG GAATCGTCGT ATCCAGCCTA CTCGCTATTG TCCTCAATGC  
3541 CGTATTAAAT CATAAAAAGA AATAAGAAAA AGAGGTGCGA GCCTCTTTTT TGTGTGACAA  
3601 AATAAAAACA TCTACCTATT CATATACGCT AGTGTCATAG TCCTGAAAAT CATCTGCATC  
3661 AAGAACAATT TCACAACCTT TATACTTTTC TCTTACAAGT CGTTCGGCTT CATCTGGATT  
3721 TTCAGCCTCT ATACTTACTA AACGTGATAA AGTTTCTGTA ATTTCTACTG TATCGACCTG  
3781 CAGACTGGCT GTGTATAAGG GAGCCTGACA TTTATATTCC CCAGAACATC AGGTTAATGG  
3841 CGTTTTTGAT GTCATTTTCG CGGTGGCTGA GATCAGCCAC TTCTTCCCG ATAACGGAGA  
3901 CCGGCACACT GGCCATATCG GTGGTCATCA TGCGCCAGCT TTCATCCCCG ATATGCACCA  
3961 CCGGGTAAAG TTCACGGGAG ACTTTATCTG ACAGCAGACG TGCACTGGCC AGGGGGATCA  
4021 CCATCCGTCG CCCGGGCGTG TCAATAATAT CACTCTGTAC ATCCACAAAC AGACGATAAC  
4081 GGCTCTCTCT TTTATAGGTG TAAACCTTAA ACTGCATTTC ACCAGTCCCT GTTCTCGTCA  
4141 GCAAAAAGAGC CGTTCATTTC AATAAACCGG GCGACCTCAG CCATCCCTTC CTGATTTTCC  
4201 GCTTTCCA

FIGURE 51C



## pDONR204 4165 bp

```
1 CGGCATTGAG GACAATAGCG AGTAGGCTGG ATACGACGAT TCCGTTTGAG AAGAACATTT
61 GGAAGGCTGT CGGTCGACTA CAGGTCACTA ATACCATCTA AGTAGTTGAA TCATAGTGAC
121 TGGATATGTT GTGTTTTACA GTATTATGTA GTCTGTTTTT TATGCAAAAT CTAATTTAAT
181 ATATTGATAT TTATATCATT TTACGTTTCT CGTTCAGCTT TTTTGTACAA AGTTGGCATT
241 ATAAAAAAGC ATTGCTTATC AATTGTGTGC AACGAACAGG TCACTATCAG TCAAAATAAA
301 ATCATTATTT GGGGCCCGAG ATCCATGCTA GCTGCAGTGC GCAGGGCCCG TGTCTCAAAA
361 TCTCTGATGT TACATTGCAC AAGATAAAAA TATATCATCA TGAACAATAA AACTGTCTGC
421 TTACATAAAC AGTAATACAA GGGGTGTTAT GAGCCATATT CAACGGGAAA CGTCTTGCTG
481 GAGGCCGCGA TTAAATTCCA ACATGGATGC TGATTTATAT GGGTATAAAT GGGCTCGCGA
541 TAATGTCGGG CAATCAGGTG CGACAATCTT TCGATTGTAT GGGGAAGCCG ATGCGCCAGA
601 GTTGTCTCTG AAACATGGCA AAGGTAGCGT TGCCAATGAT GTTACAGATG AGATGGTCAG
661 ACTAAACTGG CTGACGGAAT TTATGCCTCT TCCGACCATC AAGCATTTTA TCCGTACTCC
721 TGATGATGCA TGGTTACTCA CCACTGCGAT CCGCGGGAAA ACAGCATTCC AGGTATTAGA
781 AGAATATCCT GATTGAGGTG AAAATATTGT TGATGCGCTG GCAGTGTTCG TCGCGCCGTT
841 GCATTGCGAT CCTGTTTGTA ATTGCTCTTT TAACAGCGAT CGCGTATTTT GTCTCGCTCA
901 GCGCAATCA CGAATGAATA ACGGTTTGGT TGATGCGAGT GATTTTGATG ACGAGCGTAA
961 TGGCTGGCCT GTTGAACAAG TCTGGAAAGA AATGCATACG CTTTGTCCAT TCTCACCGBA
1021 TTCAGTCGTC ACTCATGCTG ATTTCTCACT TGATAACCTT ATTTTGTACG AGGGGAAATT
1081 AATAGGTTGT ATTGATGTTG GACGAGTCGG AATCGCAGAC CGATACCAGG ATCTTGCCAT
1141 CCTATGGAAC TGCCTCGGTG AGTTTCTCTC TTCATTACAG AAACGGCTTT TTCAAAAATA
1201 TGGTATTGAT AATCCTGATA TGAATAAATT GCAGTTTCAT TTGATGCTCG ATGAGTTTTT
1261 CTAATCAGAA TTGGTTAATT GGTGTGAACA CTGGCAGAGC ATTACGCTGA CTTGACGGGA
1321 CCGCGNCATG ACCAAAATCC CTTAACGTGA GTTTTCGTTT CACTGAGCGT CAGACCCCGT
1381 AGAAAAGATC AAAGGATCTT CTTGAGATCC TTTTTTCTG CGCGTAATCT GTGCTTGCA
1441 AACAAAAAAA CCACCGCTAC CAGCGGTGGT TTGTTTGCCG GATCAAGAGC TACCAACTCT
1501 TTTTCCGAAG GTAACGGCTC TCAGCAGAGC GCAGATACCA AATACTGTCC TTCTAGTGTA
1561 GCCGTAGTTA GGCCACCACT TCAAGAACTC TGTAGCACCG CCTACATACC TCGCTCTGCT
1621 AATCTGTGTA CCAGTGGCTG CTGCCAGTGG CGATAAGTCG TGTCTTACCG GGTGGACTC
1681 AAGACGATAG TTACCGGATA AGGCGCAGCG GTCGGGCTGA ACGGGGGGTT CGTGACACA
1741 GCCCAGCTTG GAGCGAACGA CCTACACCGA ACTGAGATAC CTACAGCGTG AGCTATGAGA
1801 AAGCGCCACG CTTCCCAGAG GGAGAAAGGC GGACAGGTAT CCGGTAAGCG GCAGGGTCGG
1861 AACAGGAGAG CGCACGAGGG AGCTTCCAGG GGGAAACGCC TGGTATCTTT ATAGTCCTGT
1921 CGGGTTTCGC CACCTCTGAC TTGAGCGTCG ATTTTGTGTA TGCTCGTCAG GGGGGCGGAG
1981 CCTATGAAA AACGCCAGCA ACGCGGCTT TTTACGGTTC CTGGCCTTTT GCTGGCCTTT
2041 TGCTCAGATG TTCTTTCTCG CGTTATCCCC TGATTCTGTG GATAACCGTA TTACCGCTAG
2101 CTGGATCGGC AAATAATGAT TTTATTTTGA CTGATAGTGA CCTGTTCGTT GCAACAAATT
2161 GATAAGCAAT GCTTTTTTAT AATGCCAACT TTGTACAAGA AAGCTGAACG AGAAACGTAA
2221 AATGATATAA ATATCAATAT ATTAAATTAG ATTTTGCATA AAAACAGAC TACATAATAC
2281 TGTAAAACAC AACATATCCA GTCATATGA TTCAACTACT TAGATGGTAT TAGTGACCTG
2341 TAGTCGACTA AGTTGGCAGC ATCACCAGAC GCACCTTTCG CCGAATAAAT ACCTGTGACG
2401 GAAGATCACT TCGCAGAATA AATAAATCCT GGTGTCCCTG TTGATACCGG GAAGCCCTGG
2461 GCCAATTTTT GGCGAAAATG AGACGTTGAT CGGCACATTT CACAACCTCT ATACTTTTCT
2521 CTTACAAGTC GTTCGGCTTC ATCTGGATTT TCAGCCTCTA TACTTACTAA ACGTGATAAA
2581 GTTTCTGTAA TTTCTACTGT ATCGACCTGC AGACTGGCTG TGTATAACGG AGCCTGACAT
2641 TTATATTCCC CAGAACATCA GGTAAATGGC GTTTTGTATG TCATTTTCGC GGTGGCTGAG
2701 ATCAGCCACT TCTTCCCCGA TAACGGAGAC CGGCACACTG GCCATATCGG TGTCATCAT
2761 GCGCCAGCTT TCATCCCCGA TATGCACCAC CGGGTAAAGT TCACGGGAGA CTTTATCTGA
2821 CAGCAGACGT GCACGTGGCA GGGGATCAC CATCCGTCGC CCGGGCGTGT CAATAATATC
2881 ACTCTGTACA TCCACAAACA GACGATAACG GCTCTCTCTT TTATAGGTGT AAACCTTAAA
2941 CTGCATTTCA CCAGTCCCTG TTCTCGTCAG CAAAAGAGCC GTTCATTTCA ATAAACGGGG
3001 CGACCTCAGC CATCCCTTCC TGATTTTCCG CTTTCCAGCG TTCGGCACGC AGACGACGGG
3061 CTTCAATTCTG CATGGTTGTG CTTACCAGAC CGGAGATATT GACATCATAT ATGCCTTGAG
3121 CAACTGATAG CTGTCGCTGT CAACTGTCAC TGTAATACGC TGCTTCATAG CACACCTCTT-
```

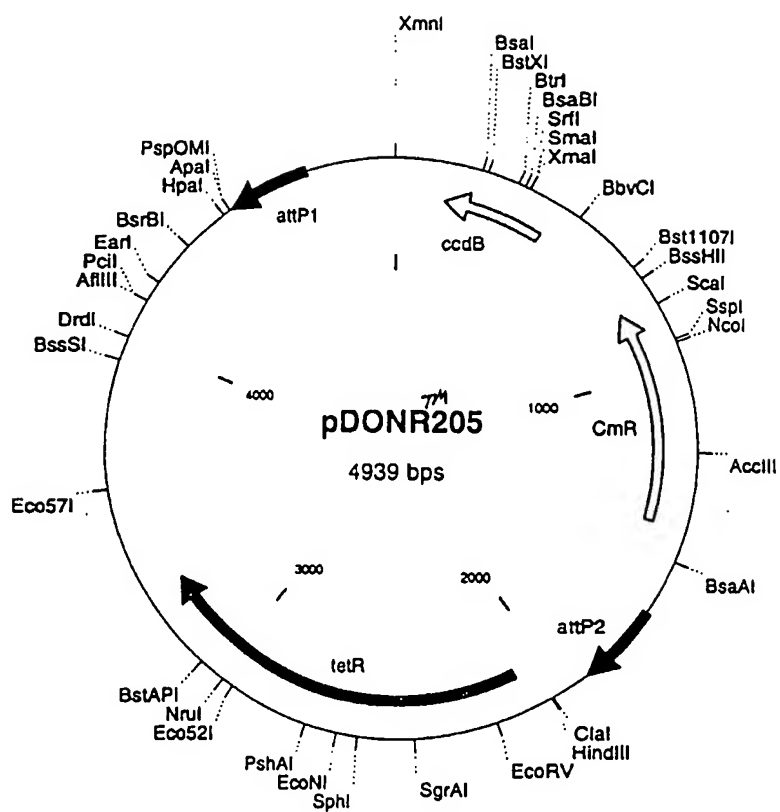


3181 TTTGACATAC TTCGGGTATA CATATCAGTA TATATTCTTA TACCGCAAAA ATCAGCGCGC  
3241 AAATACGCAT ACTGTTATCT GGCTTTTAGT AAGCCGGATC CACGCGTTTA CGCCCCGCCC  
3301 TGCCACTCAT CGCAGTACTG TTGTAATTCA TTAAGCATTG TGCCGACATG GAAGCCATCA  
3361 CAGACGGCAT GATGAACCTG AATCGCCAGC GGCATCAGCA CCTTGTCGCC TTGCGTATAA  
3421 TATTTGCCCA TGGTGA AAC GGGGGCGAAG AAGTTGTCCA TATTGGCCAC GTTTAAATCA  
3481 AAATGTTGA AACTCACCCA GGGATTGGCT GAGACGAAAA ACATATTCTC AATAAACCCCT  
3541 TTAGGGAAAT AGGCCAGGTT TTCACCGTAA CACGCCACAT CTTGCGAATA TATGTGTAGA  
3601 AACTGCCGGA AATCGTCGTG GTATTCACTC CAGAGCGATG AAAACGTTTC AGTTTGCTCA  
3661 TGGAAAACGG TGTAACAAGG GTGAACACTA TCCCATATCA CCAGCTCACC GTCTTTCATT  
3721 GCCATACGGA ATTCCGGATG AGCATTCAATC AGGCGGGCAA GAATGTGAAT AAAGGCCGGA  
3781 TAAAACTTGT GCTTATTTTT CTTTACGGTC TTTAAAAAGG CCGTAATATC CAGCTGAACG  
3841 GTCTGGTTAT AGGTACATTG AGCAACTGAC TGAAATGCCT CAAAATGTTC TTTACGATGC  
3901 CATTGGGATA TATCAACGGT GGTATATCCA GTGATTTTTT TCTCCATTTT AGCTTCCTTA  
3961 GCTCCTGAAA ATCTCGATAA CTCAAAAAAT ACGCCCGGTA GTGATCTTAT TTCATTATGG  
4021 TGAAAGTTGG AACCTCTTAC TGTTCTTGAT GCAGATGATT TTCAGGACTA TGACACTAGC  
4081 ATATATGAAT AGGTAGATGT TTTTATTTTG TCACACAAAA AAGAGGCTCG CACCTCTTTT  
4141 TCTTATTTCT TTTTATGATT TAATA

FIGURE 52C

156/240

Figure 53A: pDONR205 (tetR)



157/240

## pDONR205 4939 bp

GGCATCAGCACCTTGTGCGCTTGCCTATAATATTTGCCCATGGTGAAAACGGGGGCGAAG  
AAGTTGTCCATATTTGGCCACGTTTAAATCAAACTGGTGAACTCACCAGGGATTGGCT  
GAGACGAAAAACATATTCTCAATAAACCTTTAGGGAAATAGGCCAGGTTTTACCGTAA  
CACGCCACATCTTGCGAATATATGTGTAGAACTGCCGAAATCGTCGTGGTATTCATC  
CAGAGCGATGAAAACGTTTCAGTTTGCTCATGAAAACGGTGTAACAAGGGTGAACACTA  
TCCCATATCACCAGCTCACCGTCTTTTCATTGCCATACGGAATTCGGGATGAGCATTCCATC  
AGGCGGGCAAGAATGTGAATAAAGGCCGGATAAACTTGTGCTTATTTTTCTTTACGGTC  
TTTAAAAAGGCCGTAATATCCAGCTGAACGGTCTGGTTATAGGTACATTGAGCAACTGAC  
TGAAATGCCTCAAATGTTCTTTACGATGCCATTGGGATATATCAACGGTGGTATATCCA  
GTGATTTTTTTCTCCATTTTAGCTTCTTAGCTCCTGAAAATCTCGATAACTCAAAAAAT  
ACGCCCGGTAGTGATCTTATTTTCATTATGGTGAAAGTTGGAACCTCTTACGTGCCGATCA  
ACGTCTCATTTTCGCCAAAAGTTGGCCAGGGCTTCCCGGTATCAACAGGGACACCAGGA  
TTTATTTATTTTCGCGAAGTGATCTTCCGTACAGGTATTTATTCGGCGCAAGTGCTCG  
GGTGATGCTGCCAACTTAGTCGACTACAGGTCACTAATACCATCTAAGTAGTTGATTCAT  
AGTGACTGGATATGTTGTGTTTTACAGTATTATGTAGTCTGTTTTTTATGCAAAATCTAA  
TTTAAATATATTGATATTTATATCATTTTACGTTTCTCGTTCAGCTTTCTTGTACAAAGTT  
GGCATTTATAAGAAAGCATTGCTTATCAATTTGTTGCAACGAACAGGTCACTATCAGTCAA  
AATAAAATCATTATTTGCCATCCAGCTGCAGCTCTGGCCCGTGTCTCAAAATCTCTGATG  
TTACATTGCACAAGATAAAAAATATATCATCATGAATCTCATGTTTGACAGCTTATCATC  
GATAAGCTTTAATGCGGTAGTTTATCAGTAAATGCTAACGCAGTCAGGCACCGTGT  
ATGAAATCTAACAATGCGCTCATCGTCATCCTCGGCACCGTCACCTGGATGCTGTAGGC  
ATAGGCTTGGTTATGCCGGTACTGCCGGGCTCTTGCGGGATATCGTCCATTCCGACAGC  
ATCGCCAGTCACTATGGCGTGCTGTAGCGCTATATGCGTTGATGCAATTTCTATGCGCA  
CCCGTTCTCGGAGCACTGTCCGACCGCTTGGCCGCCGCCAGTCTCTGCTCGCTTCGCTA  
CTTGAGCCACTATCGACTACGCGATCATGGCGACCACACCCGCTCTGTGGATCCTCTAC  
GCCGAGCAGCATCGTGCCGGCATCACCGGCCACAGGTGCGGTTGCTGGCGCCTATATC  
GCCGATCATCCGATGGGGAAGATCGGGCTCGCCACTTCGGGCTCATGAGCGCTTGTTC  
GGCGTGGGTATGGTGGCAGGCCCCGTGGCCGGGGACTGTTGGGCGCCATCTCCTTGCA  
GCACCATTCCTTGCGGCGGCGGTGCTCAACGGCCTCAACCTACTACTGGGCTGCTTCCTA  
ATGCAGGAGTCGCATAAGGGAGAGCGTCGACCGATGCCCTTGAGAGCCTTCAACCCAGTC  
AGCTCCTTCCGGTGGGCGCGGGCATGACTATCGTCGCCGCCTTATGACTGTCTTCTTT  
ATCATGCAACTCGTAGGACAGGTGCCGGCAGCGCTCTGGGTCATTTTCGGCGAGGACCGC  
TTTCGCTGAGAGCGCAGATGATCGGCCTGTGCTTGGGATATCGGAATCTTGACAGCC  
CTCGCTCAAGCCTTCGTCACTGGTCCCGCCACCAACGTTTCGGCGAGAAGCAGGCGATT  
ATCGCCGGCATGGCGGCCGACGCGCTGGGCTACGTCTTGCTGGCGTTGCGACGCGAGGC  
TGGATGGCCTTCCCATATTGATTCTTCTCGCTTCCGGCGGCATCGGGATGCCCGGTTG  
CAGGCCATGCTGTCCAGGCAGGTAGATGACGACCATCAGGGACAGCTTCAAGGATCGCTC  
GCGGCTCTTACCAGCCTAACTTCGATCATTGGACCGCTGATCGTCACGGCGATTTATGCC  
GCCTCGGCGAGCACATGGAACGGGTTGGCATGGATTGTAGGCGCCGCCCTATACCTTGTC  
TGCCCTCCCCGCTTGCGTCGCGGTGCATGGAGCCGGGCCACCTCGACCTGAATGGAAGCC  
GGCGGACCTCGCTAACGGATTCAACCACTCCAAGAATTGGAGCCAATCAATTCTTGCGGA  
GAACTGTGAATGCGCAAAACCAACCTTGGCAGAACATATCCATCGCATGACCAAAATCCC  
TTAACGTGAGTTTTCTGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTC  
TTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAACAAAAAACCACCGCTACC  
AGCGGTGGTTTTGTTGCCGGATCAAGAGCTACCAACTCTTTTCCGAAGGTAAGTGGCTT  
CAGCAGAGCGCAGATACCAATACTGTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTT  
CAAGAACTCTGTAGCACCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGC  
TGCCAGTGGCGATAAGTCGTGCTTACCAGGTTGGACTCAAGACGATAGTTACCGGATAA  
GGCGCAGCGGTGGGCTGAACGGGGGTTGCTGCACACAGCCAGCTTGGAGCGAACGAC  
CTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCGAAGG  
GAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGA  
GCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCTGTGCGGTTTCGCCACCTCTGACT  
TGAGCGTCGATTTTGTGATGCTCGTCAGGGGGCGGAGCCTATGAAAAACGCCAGCAA-

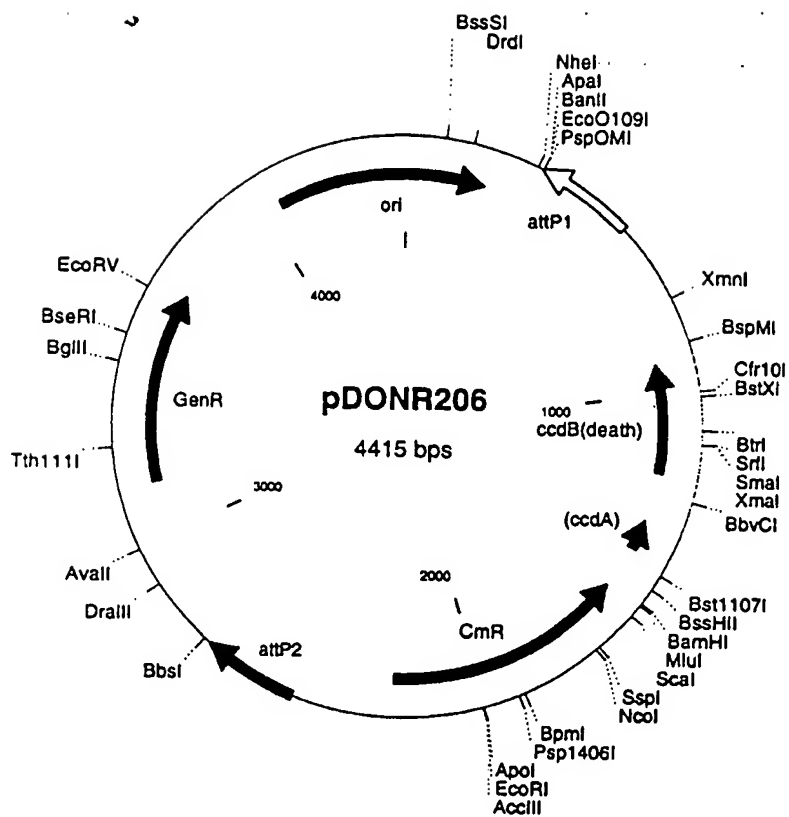
FIGURE 53B

158/240

CGCGGCCTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTGCTCACATGTTCTTCTGCTGC  
GTTATCCCTGATTCTGTGGATAACCGTATTACCGCTAGCCAGGAAGAGTTTGTAGAAAC  
GCAAAAAGGCCATCCGTCAGGATGGCCTTCTGCTTAGTTTGATGCCTGGCAGTTTATGGC  
GGGCGTCTGCCCCCACCCTCCGGGCCGTTGCTTCACAACGTTCAAATCCGCTCCCGGC  
GGATTTGTCTACTCAGGAGAGCGTTCACCGACAAACACAGATAAAACGAAAGGCCAG  
TCTTCCGACTGAGCCTTTCGTTTTATTGATGCCTGGCAGTTCCTTACTCTCGCGTTAAC  
GCTAGCATGGATCTCGGGCCCCAAATAATGATTTTATTTTGACTGATAGTGACCTGTTG  
TTGCAACAAATTGATGAGCAATGCTTTTTTATAATGCCAATTTGTACAAAAAGCTGAA  
CGAGAAACGTAATAATGATATAAATATCAATATATTAAATTAGATTTTGCATAAAAAACAG  
ACTACATAAATACTGTAAAACACAACATATCCAGTCACTATGAATCAACTACTTAGATGGT  
ATTAGTGACCTGTAGTCGACCGACAGCCTTCCAAATGTTCTTCGGGTGATGCTGCCAACT  
TAGTCGACCGACAGCCTTCCAAATGTTCTTCTCAAACGGAATCGTCGTATCCAGCCTACT  
CGCTATTGTCTCAATGCCGTATTAAATCATAAAAAGAAATAAGAAAAAGAGGTGCGAGC  
CTCTTTTTTGTGTGACAAAATAAAAAACATCTACCTATTCATATACGCTAGTGTATAGTC  
CTGAAAATCATCTGCATCAAGAACAAATTTCACAACTCTTATACTTTTCTCTTACAAGTCG  
TTCGGCTTCATCTGGATTTTCAGCCTCTATACTTACTAAACGTGATAAAGTTTCTGTAAT  
TTCTACTGTATCGACCTGCAGACTGGCTGTGTATAAGGGAGCCTGACATTTATATCCCC  
AGAACATCAGGTTAATGGCGTTTTTGATGTCATTTTCGCGGTGGCTGAGATCAGCCACTT  
CTTCCCCGATAACGGAGACCGGCACACTGGCCATATCGGTGGTCATCATGCGCCAGCTTT  
CATCCCCGATATGCACCACCGGGTAAAGTTCACGGGAGACTTTATCTGACAGCAGACGTG  
CACTGGCCAGGGGGATCACCATCCGTCGCCCCGGCGTGTCAATAATATCACTCTGTACAT  
CCACAAACAGACGATAACGGCTCTCTCTTTTATAGGTGTAAACCTTAAACTGCATTTTAC  
CAGTCCCTGTTCTCGTCAGCAAAAGAGCCGTTTCAATTTCAATAAACCGGGCGACCTCAGCC  
ATCCCTTCCTGATTTTCCGCTTTCCAGCGTTCGGCACGCAGACGACGGGCTTCATTCTGC  
ATGGTTGTGCTTACCAGACCGGAGATATTGACATCATATATGCCTTGAGCAACTGATAGC  
TGTCGCTGTCAACTGTCACTGTAAATACGCTGCTTCATAGCACACCTCTTTTTGACATACT  
TCGGGTATACATATCAGTATATATTCTTATACCGCAAAAATCAGCGCGCAAAATACGCATA  
CTGTTATCTGGCTTTTAGTAAGCCGGATCCACGCGATTACGCCCCGCCCTGCCACTCATC  
GCAGTACTGTTGTAATTCATTAAAGCATTCTGCCGACATGGAAGCCATCACAGACGGCATG  
ATGAACCTGAATCGCCAGC

FIGURE 53C

159/240



160/240

## pDONR206 4415 bp

CGGCATTGAGGACAATAGCGAGTAGGCTGGATACGACGATTCCGTTTGAGAAGAACATTT  
GGAAGGCTGTGGTTCGACTACAGGCTCACTAATACCATCTAAGTAGTTGAATCATAGTGAC  
TGGATATGTTGTGTTTTACAGTATTATGTAGTCTGTTTTTTATGCAAAATCTAATTTAAT  
ATATTGATATTTATATCATTTTACGTTTCTCGTTCAGCTTTTTTGTACAAAGTTGGCATT  
ATAAAAAAGCATTGCTTATCAATTTGGTGTGCAACGAACAGGTCATATCAGTCAAAATAAA  
ATCATTATTTGGGGCCGAGATCCATGCTAGCGGTAATACGGTTATCCACAGAATCAGGG  
GATAACGCAGGAAAGAATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAG  
GCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCAAAAAATCGA  
CGTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCT  
GGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCC  
TTTCTCCCTTCGGGAAGCGTGCGCTTTCTCATAGCTCAGCTGTAGGTATCTCAGTTCTG  
GTGTAGGTCGTTGCTTCAAGCTGGGCTGTGTGCAAGAACCCCGTTTCAGCCGACCGC  
TGCGCCTTATCCGGTAACATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCA  
CTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAG  
TTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCT  
CTGCTGAAGCCAGTTACCTTCGGAAGAGAGTTGGTAGCTCTTGATCCGGCAAAACAAACC  
ACCGCTGGTAGCGGTGGTTTTTTTGTGTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGA  
TCTCAAGAAGATCCTTTGATCTTTTCTACGGGCTCTGACGCTCAGTGGAACGAAACTCA  
CGTTAAGGGATTGTTGGTCATGNCGCCGTCCTCCGTCAGTCAAGTCAGCGTAATGCTCTGCCAGTGT  
TACAACCAATTAACCAATTCTGATTAGAAAACTCATCGAGCATCAAATGAAACTGCAAT  
TTATTCATATCAGGATTATCAATACCATATTTTGAAGAACCGGTTTCTGTAATGAAGGA  
GAAACTCACCGAGGCAGTTCCATAGGATGGCAAGATCCTGGTATCGGTCTGCGATTCCG  
ACTCGTCCAACATCAATACACCTATTAGCCGAGGTCTTCCGATCTCCTGAAGCCAGGGC  
AGATCCGTGCACAGCACCTTGCCGTAGAAGAACAGCAAGGCCGCAATGCCTGACGATGC  
GTGGAGACCGAAACCTTGCGCTCGTTGCGCCAGCCAGGACAGAAATGCCTCGACTTCGCTG  
CTGCCAAGGTTGCCGGGTGACGCACACCGTGGAACGGATGAAGGCACGAACCCAGTTG  
ACATAAGCCTGTTCCGGTTCGTAAACTGTAATGCAAGTAGCGTATGCGCTCACGCAACTGG  
TCCAGAACCTTGACCGAACGCAGCGGTGGTAACGGCGCAGTGGCGGTTTTTCATGGCTTGT  
TATGACTGTTTTTTGTACAGTCTATGCCTCGGGCATCCAAGCAGCAAGCGCGTTACGCC  
GTGGTTCGATGTTTGTATGTTATGGAGCAGCAACGATGTTACGCAGCAGCAACGATGTTAC  
GCAGCAGGGCAGTCGCCCTAAACAAAGTTAGGTGGCTCAAGTATGGGCATCATTGCGAC  
ATGTAGGCTCGGCCCTGACCAAGTCAAATCCATGCGGGCTGCTCTGATCTTTTCGGTCTG  
TGAGTTCGGAGACGTAGCCACCTACTCCCAACATCAGCCGGACTCCGATTACCTCGGGAA  
CTTGCTCCGTAGTAAGACATTCATCGCGCTTGCTGCCTTCGACCAAGAAGCGGTTGTTGG  
CGCTCTCGCGGCTTACGTTCTGCCCAGGTTTGAGCAGCCGCGTAGTGAGATCTATATCTA  
TGATCTCGCAGTCTCCGGCAGCACCGGAGGCAGGGCATTGCCACCGCGCTCATCAATCT  
CCTCAAGCATGAGGCCAACGCGCTTGGTCTTATGTGATCTACGTGCAAGCAGATTACGG  
TGACGATCCCGCAGTGGCTCTCTATACAAAGTTGGGCATACGGGAAGAAGTGATGCACTT  
TGATATCGACCCCAAGTACGCCACCTAACAAATTCGTTCAAGCCGAGATCGGCTTCCGGC  
CTAATTTCCCTCTGTCAAAAAATAAGGTTATCAAGTGAGAAATCACCATGAGTGACGACTG  
AATCCGGTGAGAATGGCAAAAGCGTATGCATTTCTTTCCAGACTTGTTCAACAGGCCAGC  
CATTACGCTCGTCATCAAAATCACTCGCATCAACCAACCGTTATTCATTCTGTGATTGCG  
CCTGAGCGAGACGAAATACGCGATCGCTGTTAAAAGGACAATTACAAACAGGAATCGAAT  
GCAACCGGCGCAGGAACACTGCCAGCGCATCAACAATATTTTCACTGAATCAGGATATT  
CTTCTAATACCTGGAATGCTGTTTTCCCGCGGATCGCAGTGGTGAGTAACCATGCATCAT  
CAGGAGTACGGATAAAATGCTTGATGGTTCGGAAGAGGCATAAATCCGTGACCCAGTTTA  
GTCTGACCATCTCATCTGTAAACATCATTGGCAACGCTACCTTTGGCATGTTTCAGAAACA  
ACTCTGGCGCATCGGGCTTCCCATACAATCGAAAGATTGTGCGACCTGATTGCCCCGACAT  
TATCGCGAGCCCATTTATACCCATATAAATCAGCATCCATGTTGGAATTTAATCGCGGCC  
TCCAGCAAGACGTTTCCCGTTGAATATGGCTCATAACACCCCTTGATTACTGTTTATGT  
AAGCAGACAGTTTTATTGTTTCATGATGATATATTTTTATCTTGTGCAATGTAAACATCAGA  
GATTTTGAGACACGGGCCCNGCGCACTGCAGCTGGATCGGCAAAATAATGATTTTATTTTG  
ACTGATAGTGACCTGTTGTTGCAACAAATGATAAGCAATGCTTTTTTATAATGCCAAC -

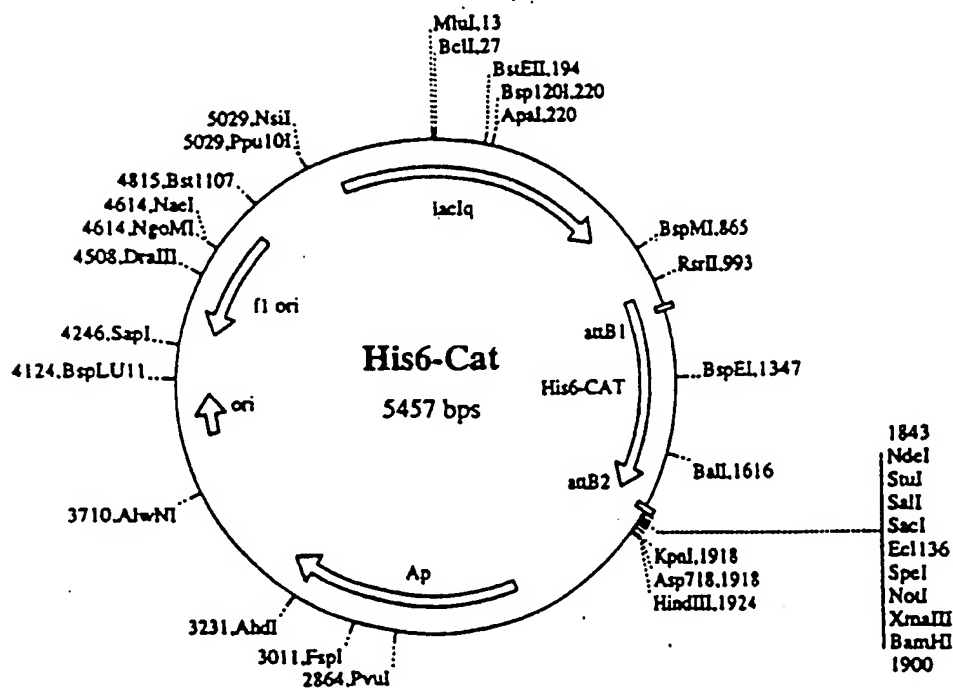
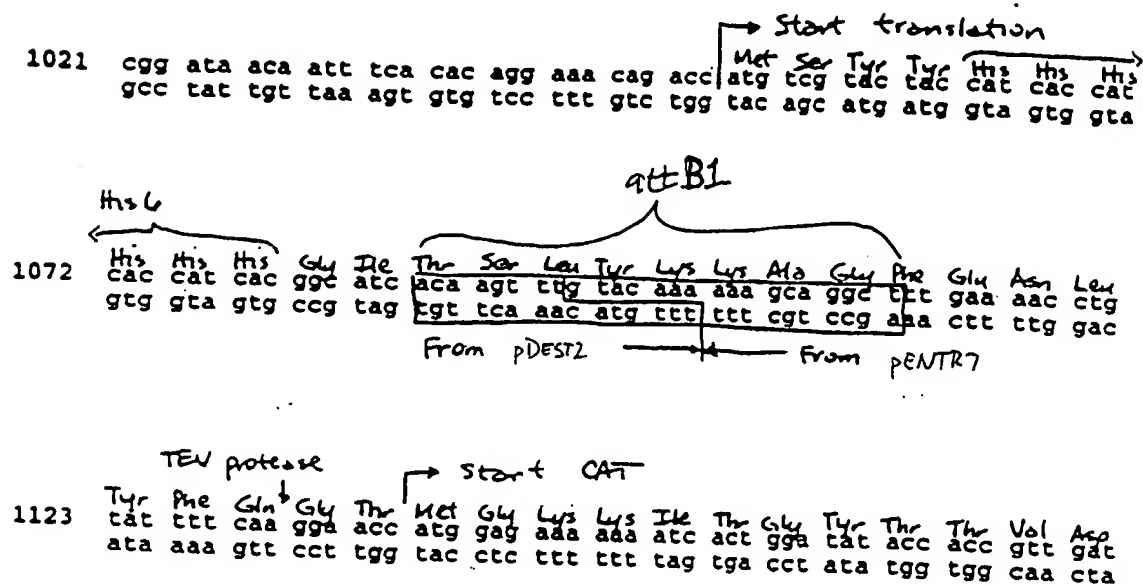
FIGURE 54B

161/240

TTTGTACAAGAAAGCTGAACGAGAAACGTAAAATGATATAAATATCAATATATTAAATTA  
GATTTTGCATAAAAAACAGACTACATAATACTGTAAAACACAACATATCCAGTCACTATG  
ATTCAACTACTTAGATGGTATTAGTGACCTGTAGTCGACTAAGTTGGCAGCATCACCCGA  
CGCACTTTGCGCCGAATAAATACCTGTGACGGAAGATCACTTCGCAGAATAAATAAATCC  
TGGTGTCCCTGTTGATACCGGAAGCCCTGGGCGCAACTTTTGGCGAAAATGAGACGTTGA  
TCGGCAGCTAAGAGGTTCCAACCTTTACCATAATGAAATAAGATCACTACCGGGCGTATT  
TTTTGAGTTATCGAGATTTTCAGGAGCTAAGGAAGCTAAAATGGAGAAAAAATCACTGG  
ATATACCCACCGTTGATATATCCCAATGGCATCGTAAAGAACATTTTGAGGCATTTTCAGTC  
AGTTGCTCAATGTACCTATAACCAGACCGTTTCAGCTGGATATTACGGCCTTTTAAAGAC  
CGTAAAGAAAAATAAGCACAGTTTTATCCGGCCTTTATTTCACATTCTTGCCCGCTGAT  
GAATGCTCATCCGAATTCGGTATGGCAATGAAAGACGGTGAGCTGGTGATATGGGATAG  
TGTTACCCCTTGTTACACCGTTTTCCATGAGCAAACTGAAACGTTTTTCATCGCTCTGGAG  
TGAATACCACGACGATTTCCGGCAGTTTCTACACATATATTCCGAAGATGTGGCGTGTTA  
CGGTGAAAACCTGGCCTATTTCCCTAAAGGGTTTTATTGAGAATATGTTTTTCGTCTCAGC  
CAATCCCTGGGTGAGTTTTCACCACTTTGATTTAAACGTGGCCAATATGGACAACCTTCTT  
CGCCCCCGTTTTTACCATGGGCAATATTATACGCAAGGCGACAAGGTGCTGATGCCGCT  
GGCGATTGAGGTTTCATCATGCCGTCTGTGATGGCTTCCATGTCCGGCAGAATGCTTAATGA  
ATTACAACAGTACTGCGATGAGTGGCAGGGCGGGGCGTAAACGCGTGGATCCGGCTTACT  
AAAAGCCAGATAACAGTATGCGTATTTGCGCGCTGATTTTTGCGGTATAAGAATATATAC  
TGATATGTATACCCGAAGTATGTCAAAAAGAGGTGTGCTATGAAGCAGCGTATTACAGTG  
ACAGTTGACAGCGACAGCTATCAGTTGCTCAAGGCATATATGATGTCAATATCTCCGGTC  
TGGTAAGCACAAACCATGCAGAATGAAGCCCGTCGTCTGCGTGCCGAACGCTGGAAGCGG  
AAAATCAGGAAGGGATGGCTGAGGTGCGCCCGTTTATTGAAATGAACGGCTCTTTTGCTG  
ACGAGAACAGGGACTGGTGAAATGCAGTTTAAAGTTTACACCTATAAAGAGAGAGCCGT  
TATCGTCTGTTTGTGGATGTACAGAGTGATATTATTGACACGCCCCGGGCGACGGATGGTG  
ATCCCCCTGGCCAGTGACAGTCTGCTGTGTCAGATAAAGTCTCCCGTGAACCTTACCCGGTG  
GTGCATATCGGGGATGAAAGCTGGCGCATGATGACCACCGATATGGCCAGTGTGCCGGTC  
TCCGTTATCGGGGAAGAAGTGGCTGATCTCAGCCACCGCGAAAATGACATCAAAAACGCC  
ATTAACCTGATGTTCTGGGGAATATAAATGTCAGGCTCCGTTATACACAGCCAGTCTGCA  
GGTCGATACAGTAGAAATTACAGAACTTTATCACGTTTATGTAAGTATAGAGGCTGAAAA  
TCCAGATGAAGCCGAACGACTTGTAAGAGAAAAGTATAAGAGTTGTGAAATTGTTCTTGA  
TGCAGATGATTTTCAGGACTATGACACTAGCATATATGAATAGGTAGATGTTTTTATTTT  
GTCACACAAAAAAGAGGCTCGCACCTCTTTTTCTTATTTCTTTTATGATTTAATA

FIGURE 54 C

Figure 55 An Entry (pENTR7) Clone of CAT Subcloned into pDEST2





163/240

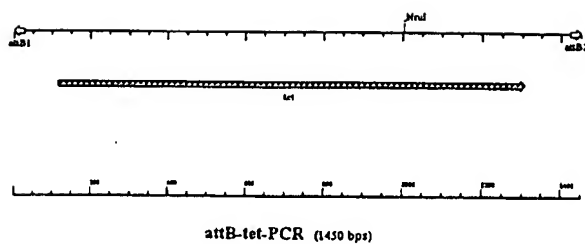
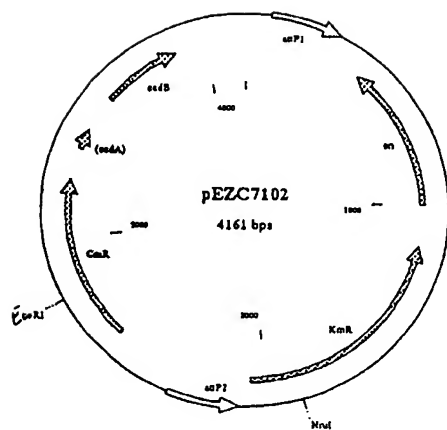


FIGURE 56

164/240

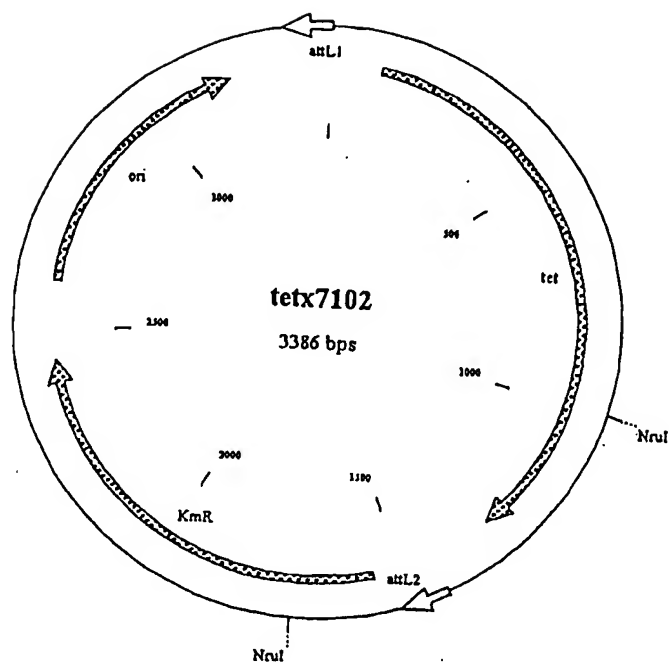


FIGURE 57

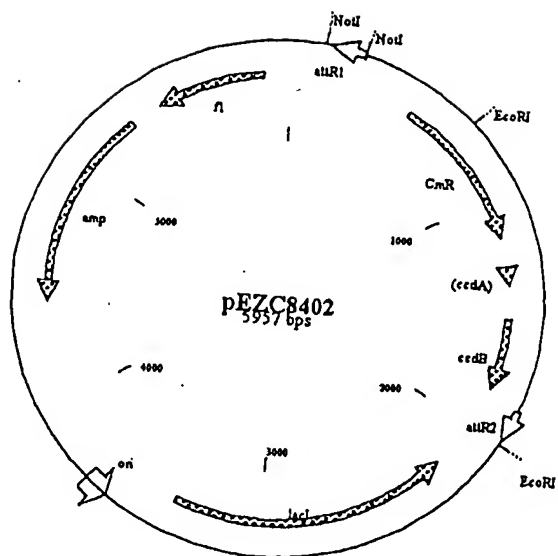


FIGURE 58

166/240

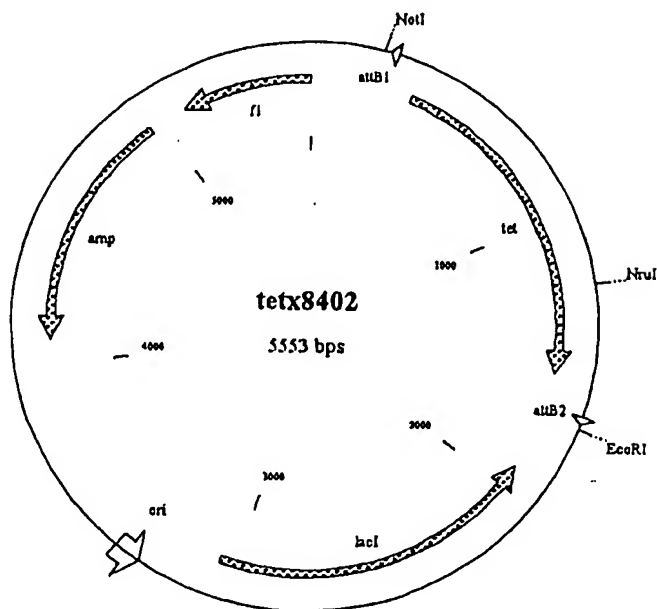


FIGURE 59

167/240

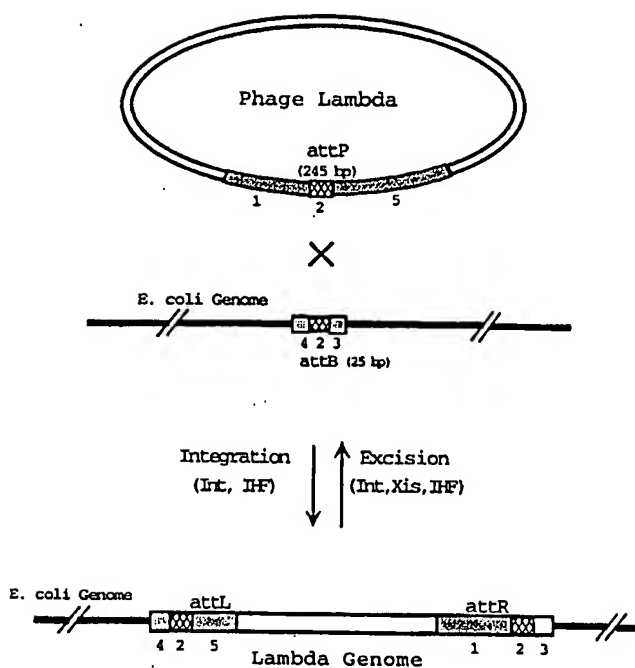


FIGURE 60

168/240

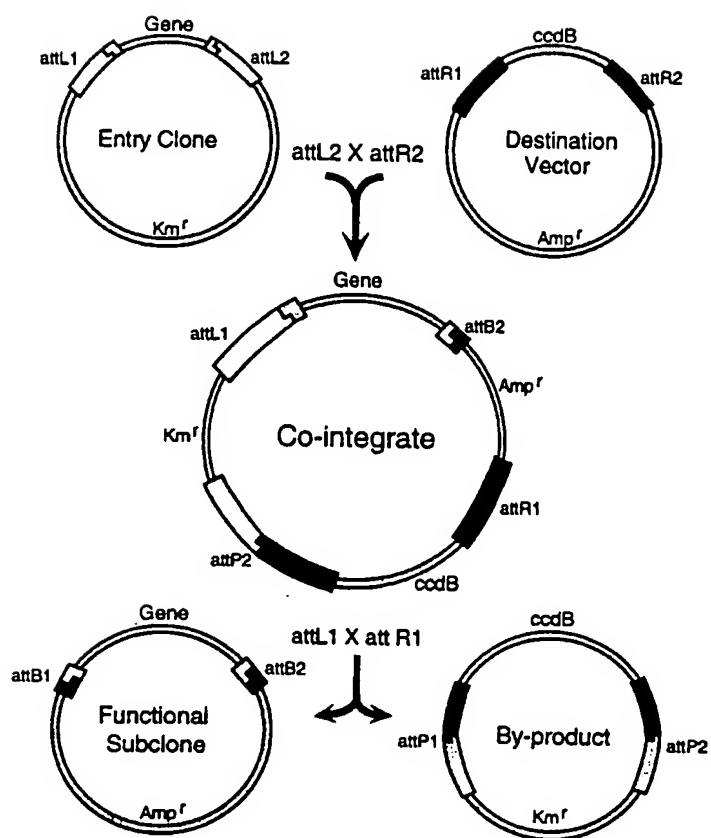


FIGURE 61

169/240

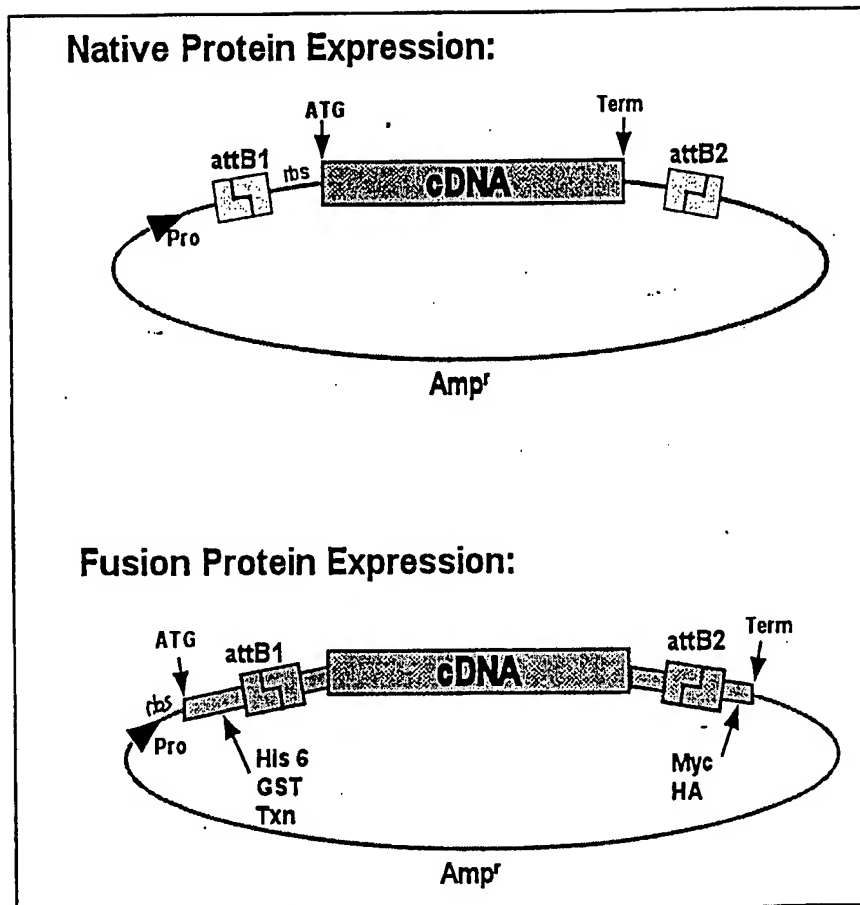


FIGURE 62

170/240

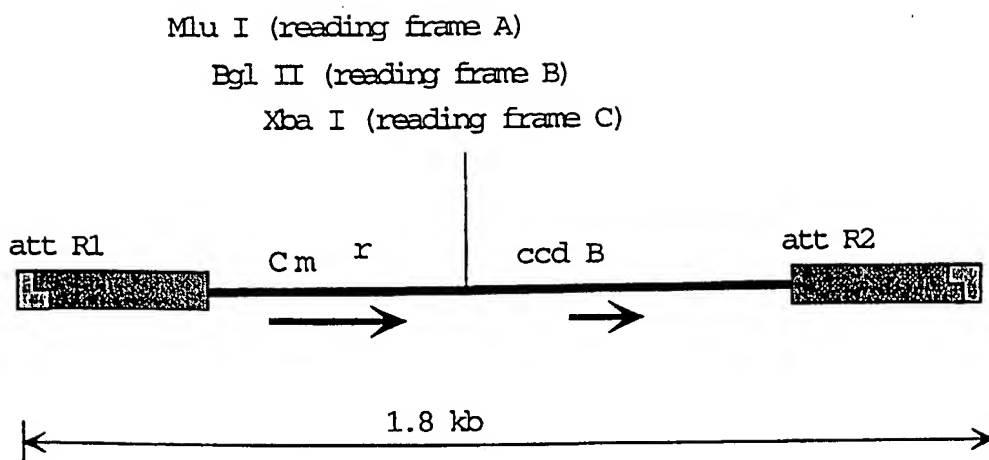
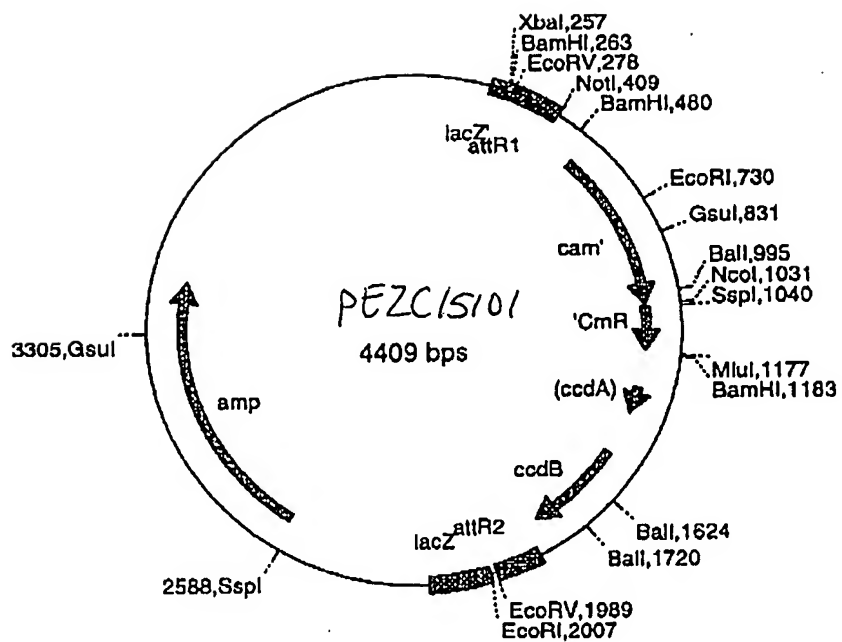


FIGURE 63



171/240

FIGURE 64A



172/240

FIGURE 6A

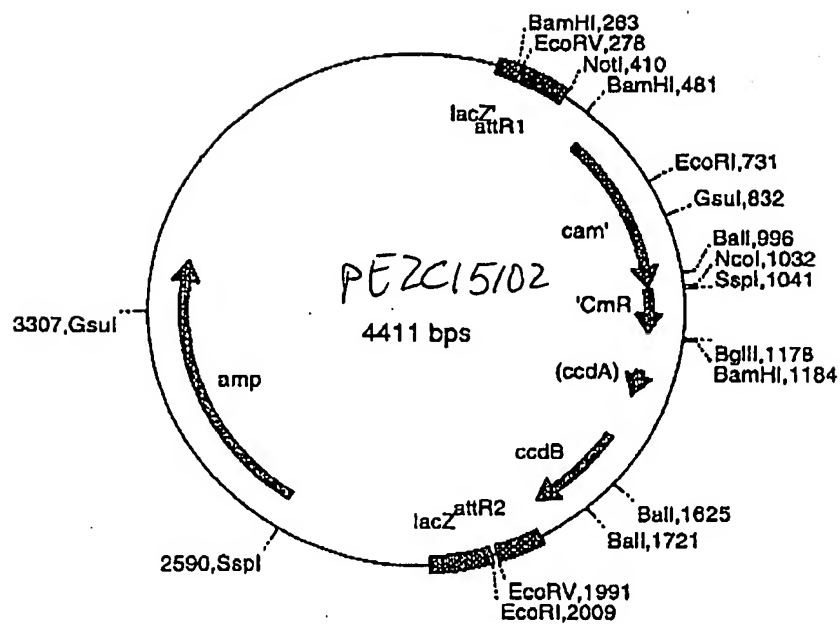
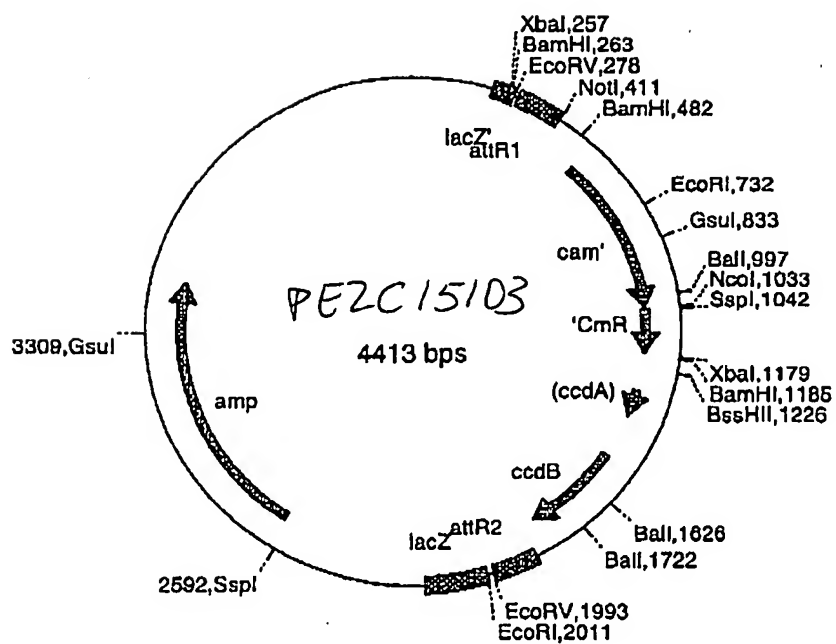
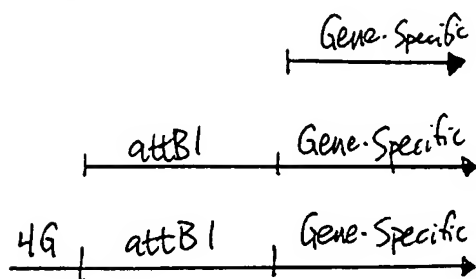


FIGURE 64C



# Primers for Amplifying *tetR* and *ampR* for Cloning by Recombination

## Primers



## Reverse Primers

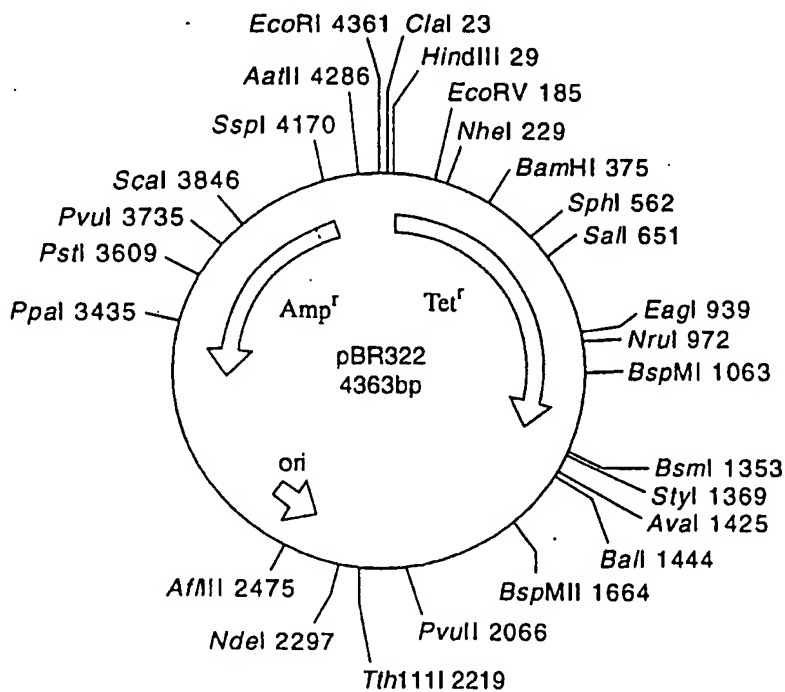
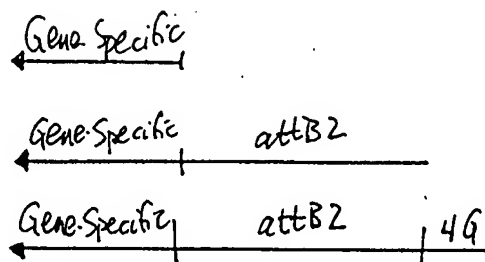


FIGURE 65

**Results of Cloning  
tet and amp PCR Products  
by Recombination**

PCR Product Used in GCS Reactions	No. Colonies Obtained (100 $\mu$ l plated)	Form of DNA Analyzed	Colonies Obtained of Predicted Size
tet	6, 10	SC	0 of 8
attB-tet	9, 6	SC	1 of 8
attB+4G-tet	824, 1064	SC AvaI+Bam	7 of 7 7 of 7
amp	7, 13	SC	0 of 8
attB-amp	18, 22	SC	3 of 8
attB+4G-amp	3020, 3540	SC PstI	8 of 8 8 of 8
attB Plasmid (Pos. Control)	320, 394		

FIGURE 66

176/240

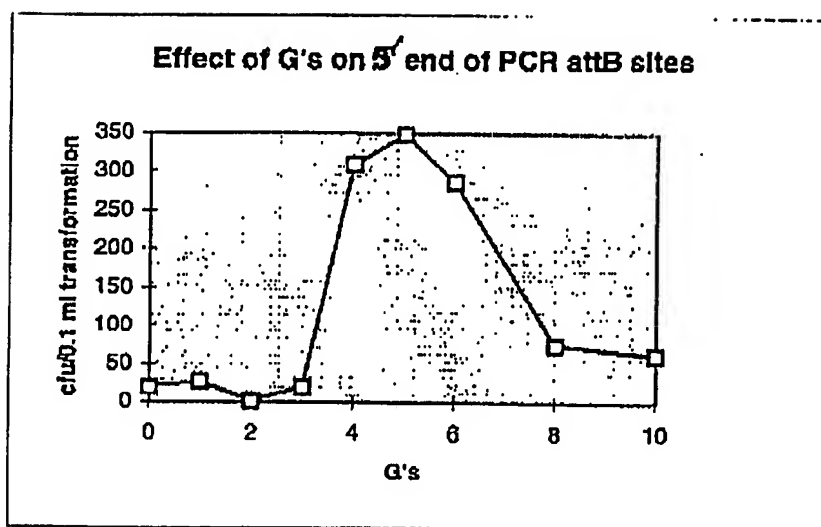


FIGURE 67

177/260

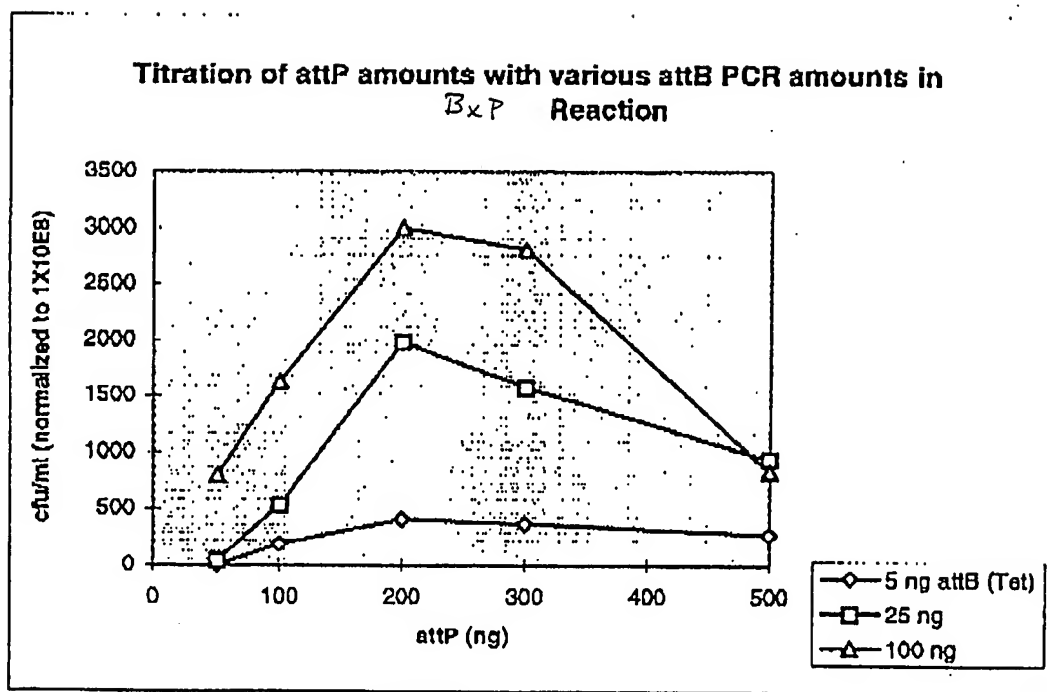
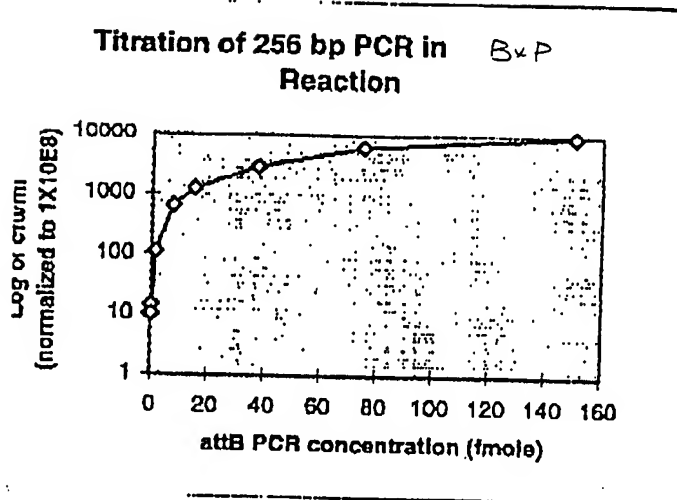


FIGURE 68

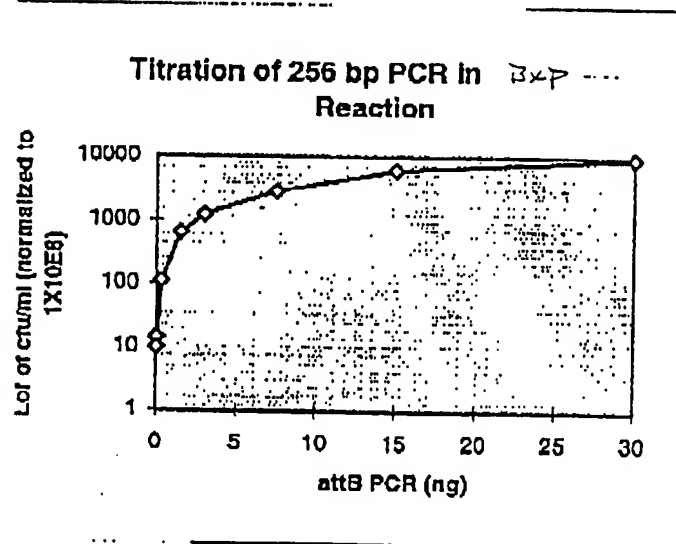
178/240

FIGURE  
69

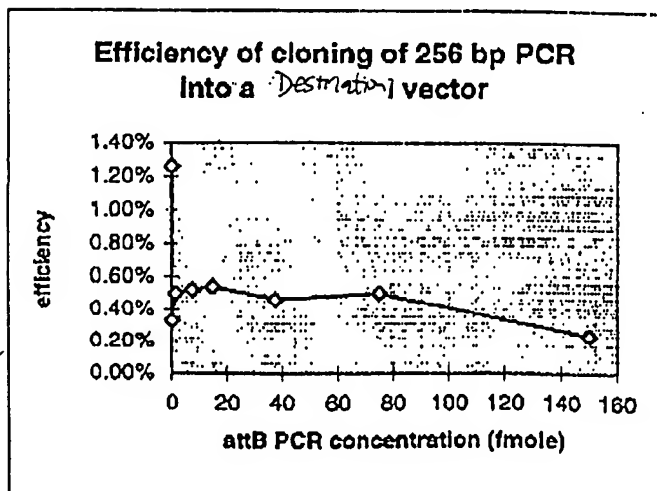
A



B



C

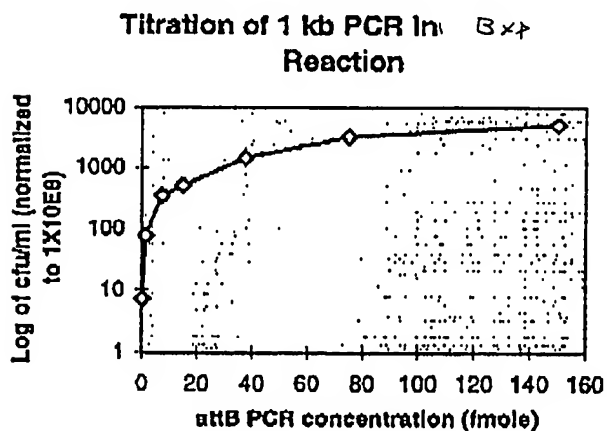




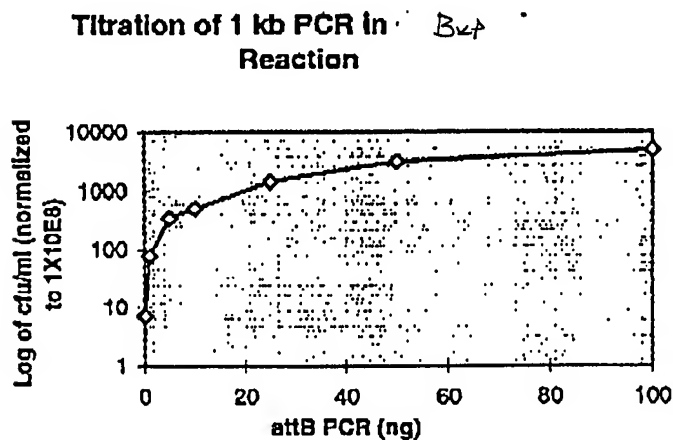
179/240

FIGURE  
70

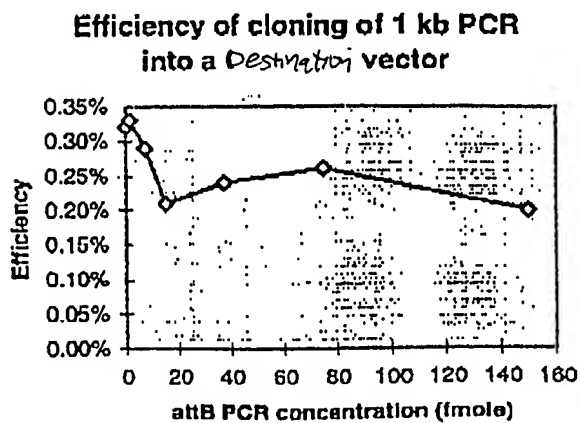
A



B



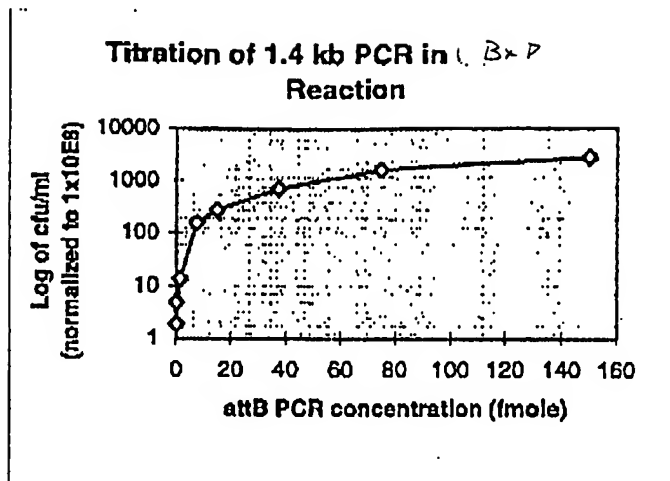
C



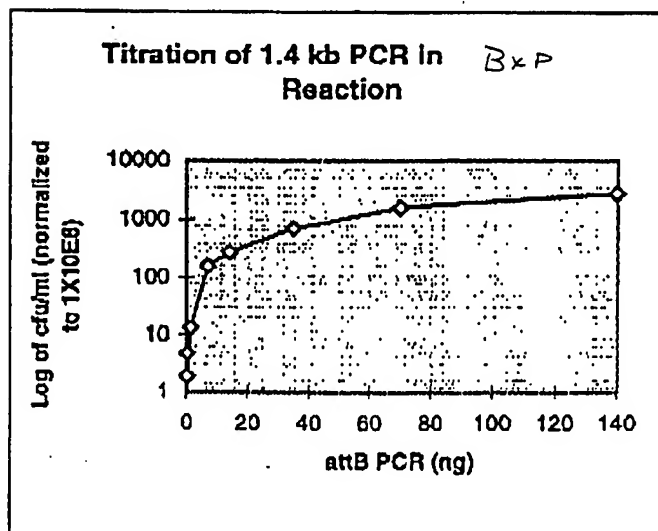
180/240

FIGURE 71

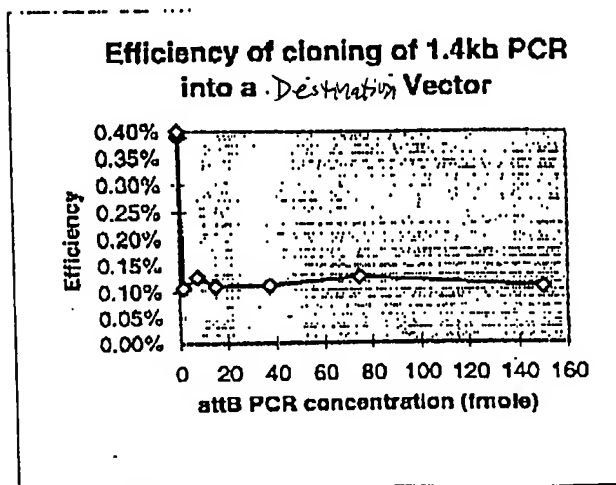
A



B



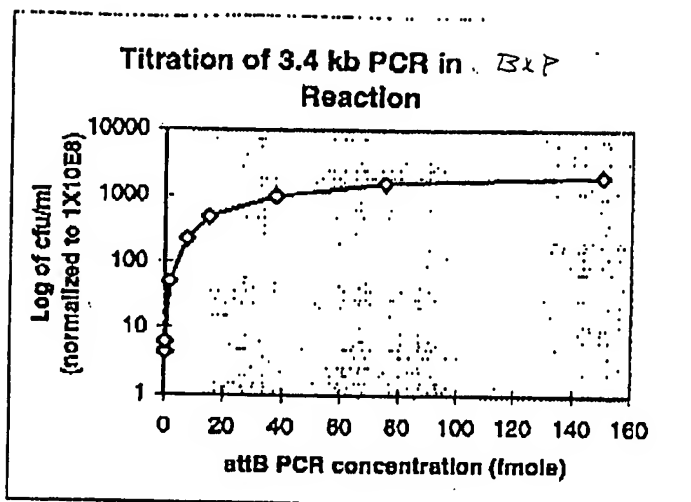
C



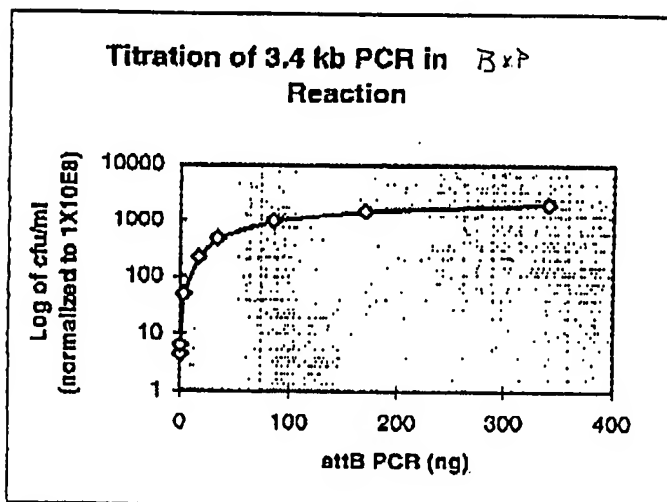
181/240

FIGURE 72

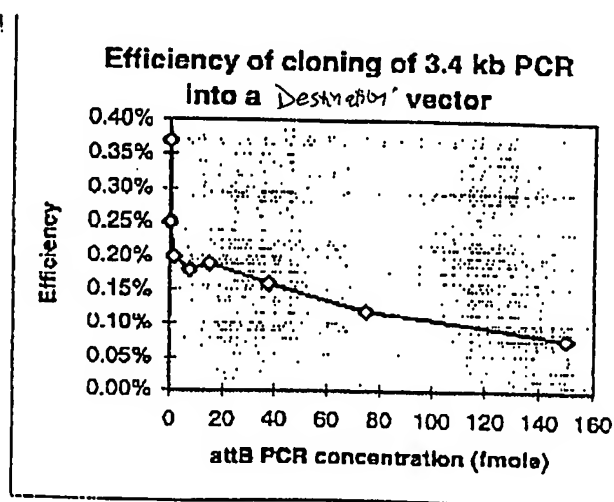
A



B



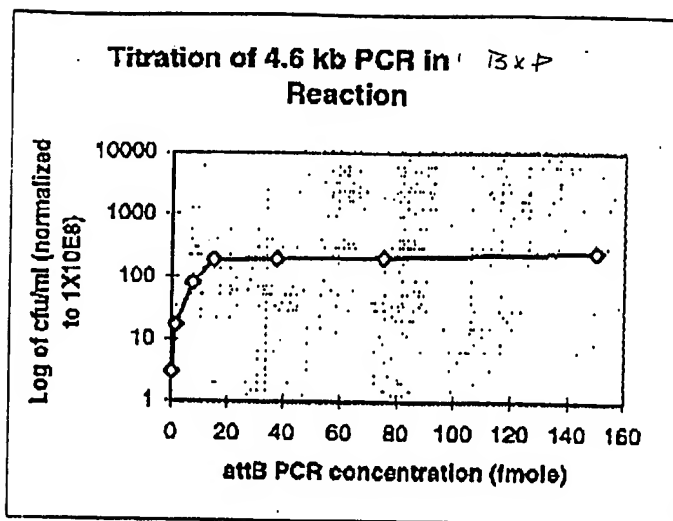
C



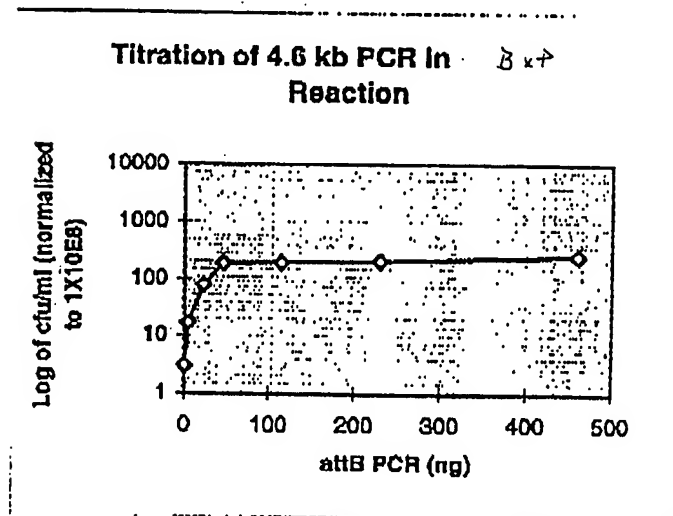
182/240

FIGURE 73

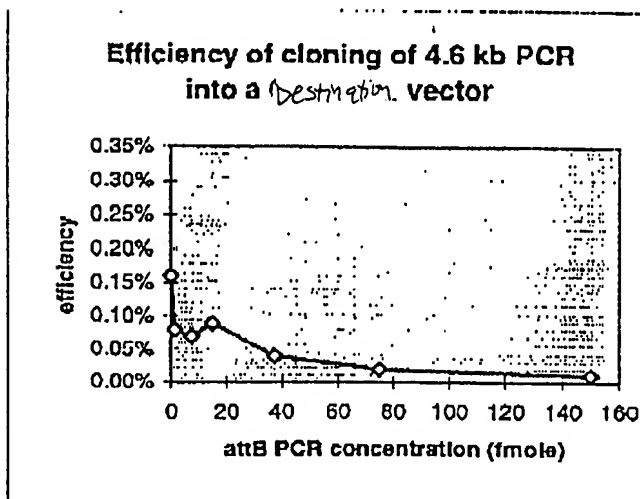
A



B



C



6.9 kb PCR DNA Titration in  $\alpha$  B $\times$ P Reaction

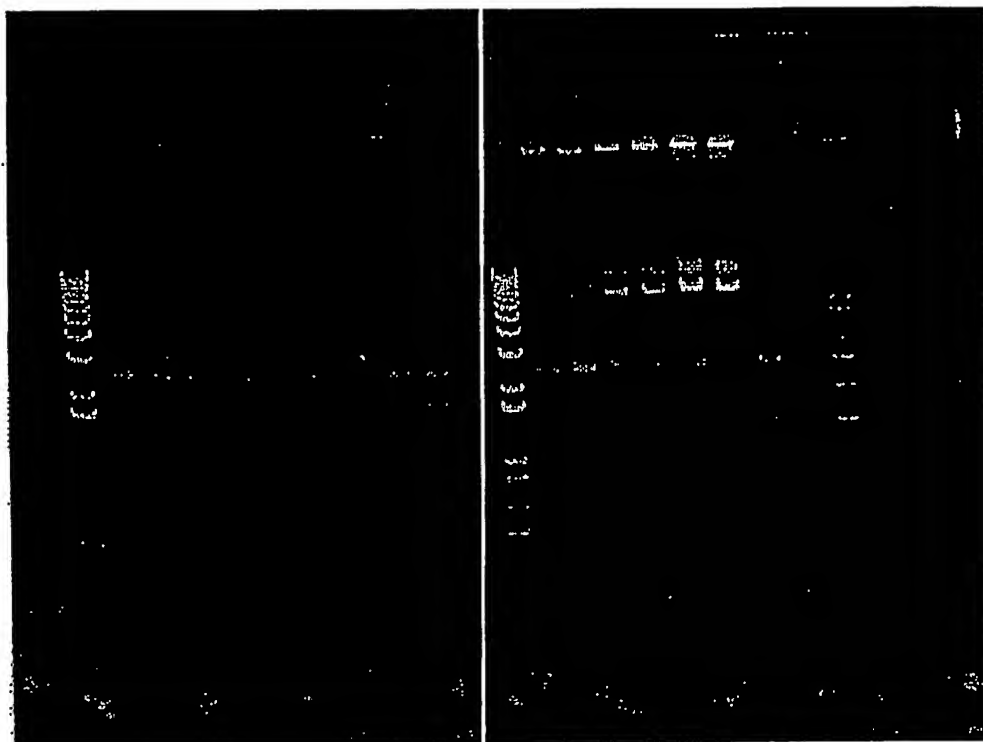


FIGURE 74

184/240

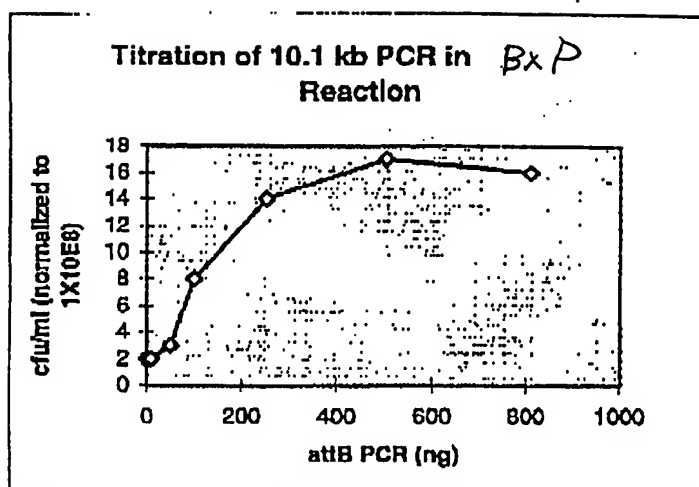


FIGURE 75-

# 10.1 kb PCR DNA Titration in BxP Reaction

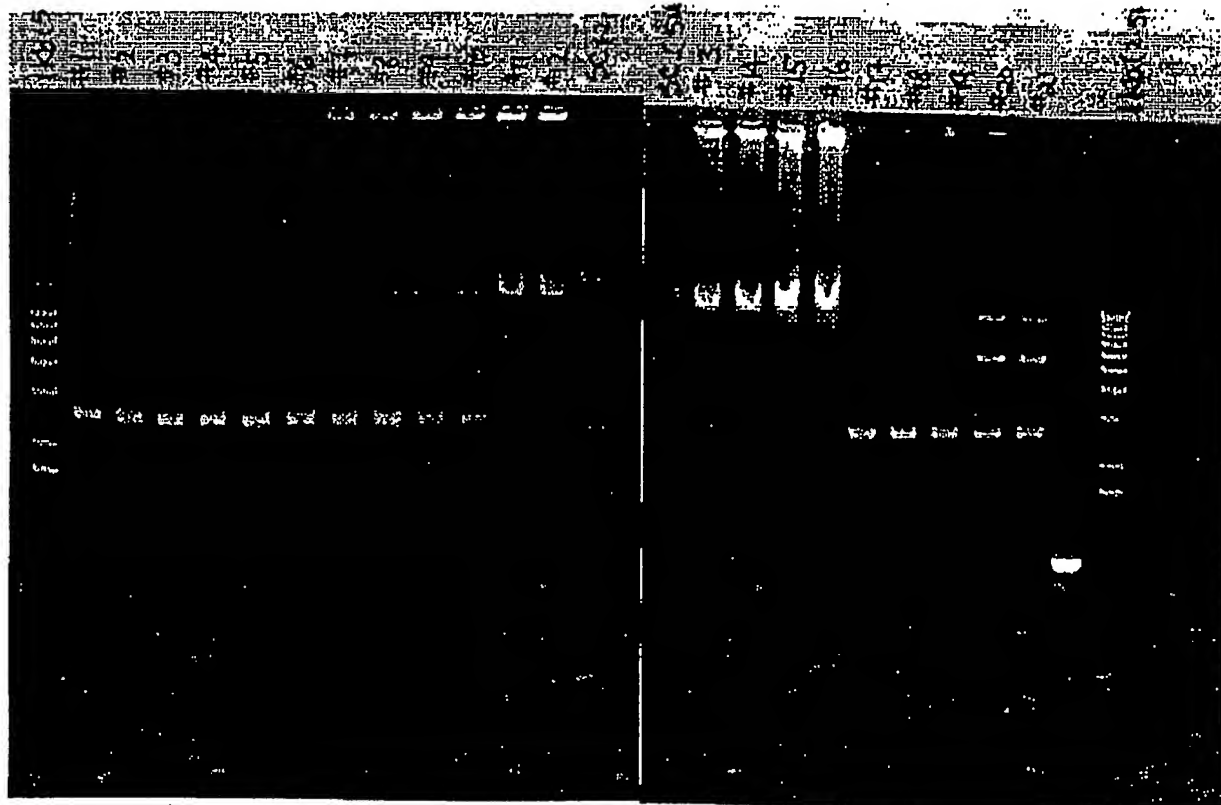


FIGURE 76

### Cloning of PCR Products of Different Sizes with the GATEWAY™ PCR Cloning System

Size	fmols PCR DNA	ng PCR DNA	Cols/ml Transformation (pUC=10 <sup>8</sup> CFU/ml)	Correct Clones/Total Examined**
0.26 kb*	15 37.5	3 7.5	1223 2815	10/10 (a)
1.0 kb	15 37.5	10 25	507 1447	49/50 (b)
1.4 kb	15 37.5	14 35	271 683	48/50 (c)
3.4 kb	15 37.5	34 85	478 976	9/10 (a)
4.6 kb	15 37.5	46 115	190 195	10/10 (a)
6.9 kb	15 37.5	69 173	30 (235)** 54 (463)**	47/50 (b)

\*The 0.26 kb PCR product was used unpurified; all the others were purified by precipitation with PEG/MgCl<sub>2</sub> as described in the text of Example 9, to remove primer dimers potentially present. Standard incubations were for 60 min.

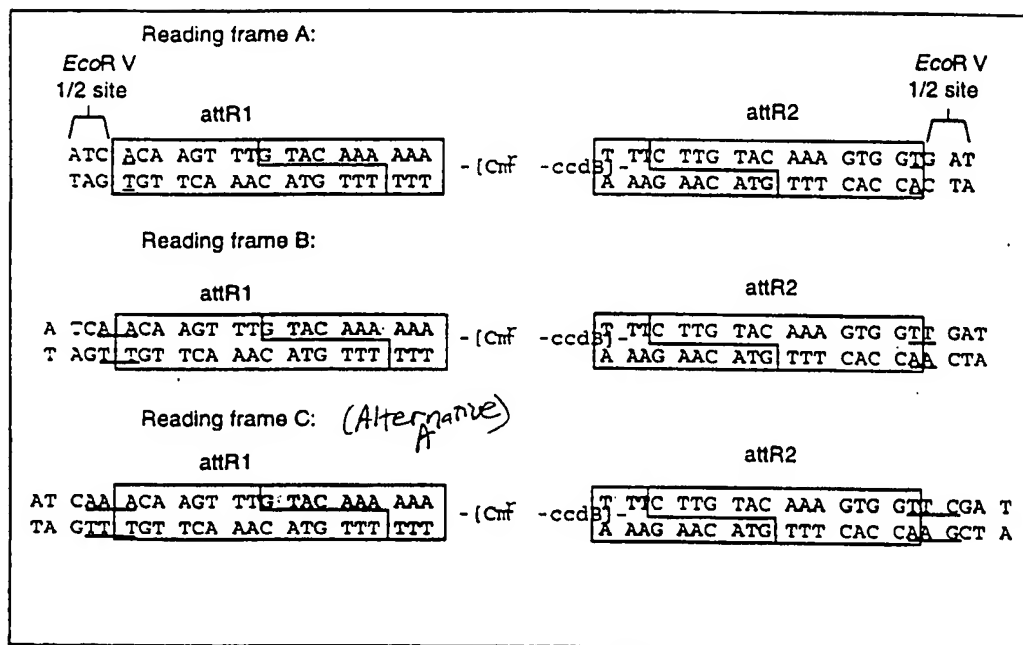
\*\*overnight incubation

- (a) DNA minipreps
- (b) ampR/kanR
- (c) tetR/kanR

**Figure 77**



187/240



Reading frame C: (Alternative)  
B

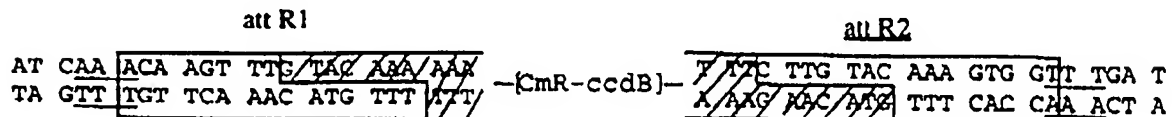


FIGURE 78

188/260

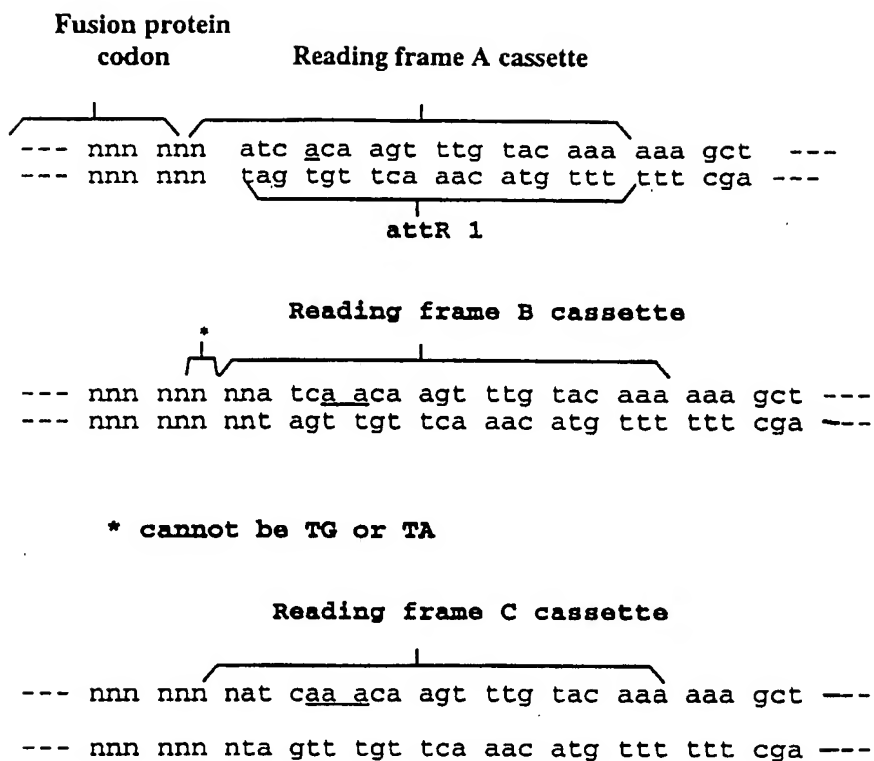


FIGURE 79



190/240

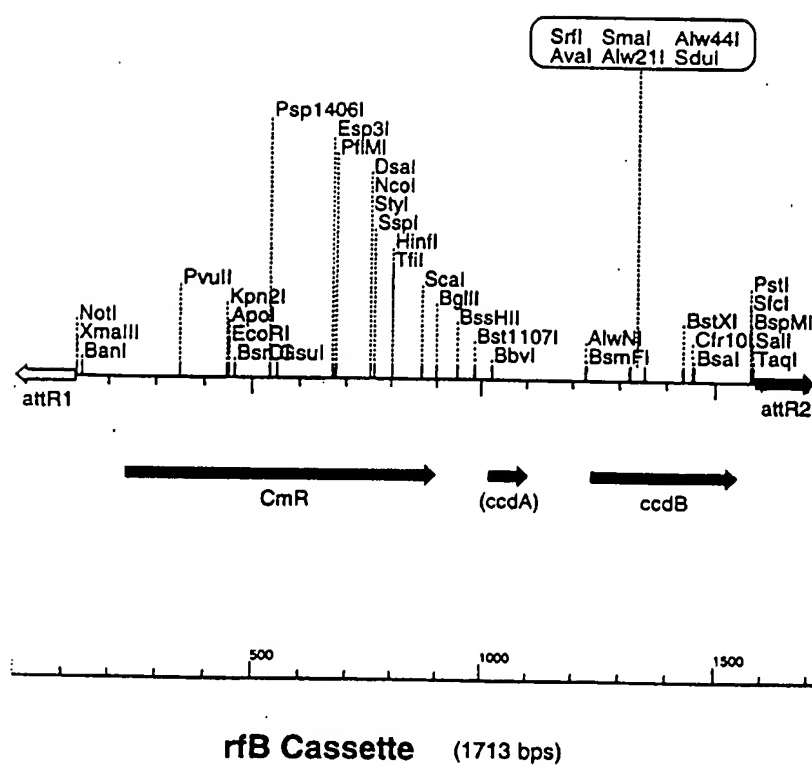
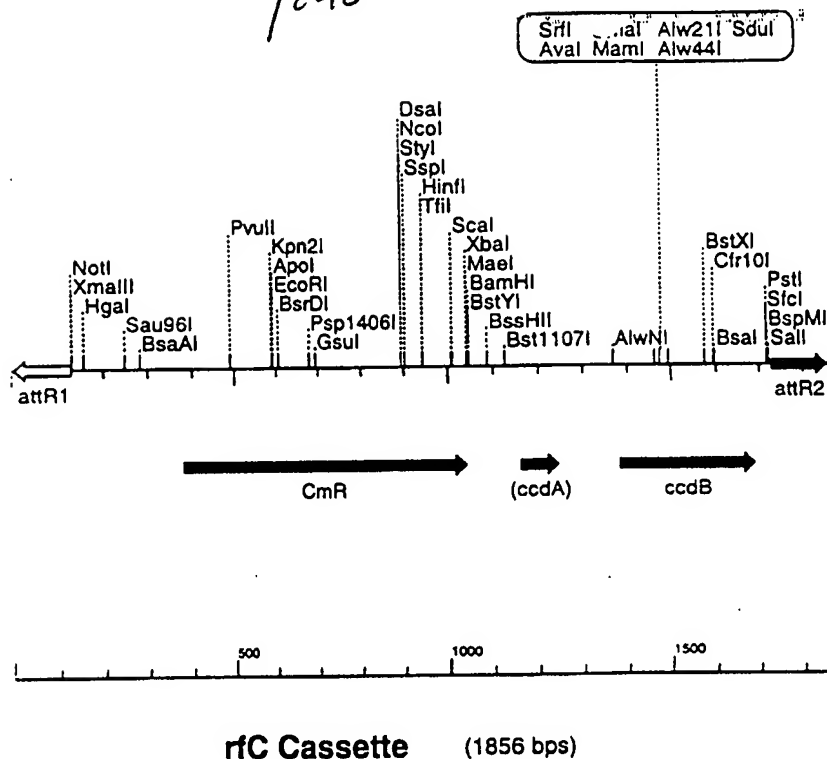


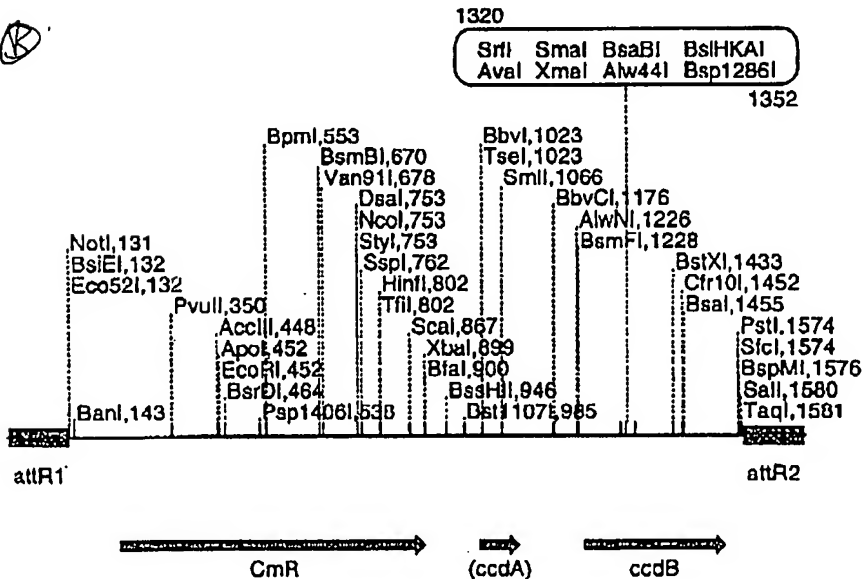
FIGURE 81

191/240

(A)



(B)



rC cassette (1715 bps)

FIGURE 82

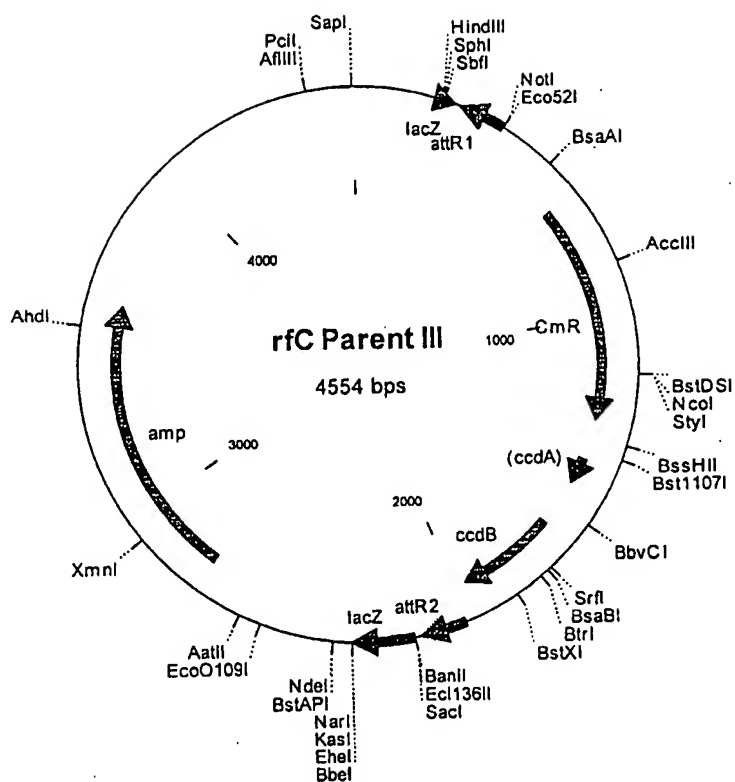


FIGURE 83A

## prfC Parent III 4554 bp

<u>Location (Base Nos.)</u>	<u>Gene Encoded</u>
410..286	attR1
660..1319	CmR
1439..1523	inactivated ccdA
1661..1966	ccdB
2007..2131	attR2
2753..3613	amp

```

1 GCGCCCAATA CGCAAACCGC CTCTCCCCGC GCGTTGGCCG ATTCATTAAT GCAGCTGGCA
61 CGACAGGTTT CCCGACTGGA AAGCGGGCAG TGAGCGCAAC GCAATTAATG TGAGTTAGCT
121 CACTCATTAG GCACCCAGG CTTTACACTT TATGCTTCCG GCTCGTATGT TGTGTGGAAT
181 TGTGAGCGGA TAACAATTC ACACAGGAAA CAGCTATGAC CATGATTACG CCAAGCTTGC
241 ATGCCTGCAG GTCGACTCTA GAGGATCCCC GGTACCGAT ATCAAACAAG TTTGTACAAA
301 AAAGCTGAAC GAGAAACGTA AAATGATATA AATATCAATA TATTAAATTA GATTTTGCAT
361 AAAAAACAGA CTACATAATA CTGTAAAACA CAACATATCC AGTCACTATG GCGGCCGCTA
421 AGTTGGCAGC ATCACCCGAC GCACCTTTCG CCGAATAAAT ACCTGTGACG GAAGATCACT
481 TCGCAGAATA AATAATCCT GGTGTCCCTG TTGATACCGG GAAGCCCTGG GCCAACTTTT
541 GCGGAAAATG AGACGTTGAT CGGCACGTAA GAGGTTCCAA CTTTCACCAT AATGAAATAA
601 GATCACTACC GGGCGTATTT TTTGAGTTAT CGAGATTTTC AGGAGCTAAG GAAGCTAAAA
661 TGGAGAAAAA AATCACTGGA TATACCACCG TTGATATATC CCAATGGCAT CGTAAAGAAC
721 ATTTTGAGGC ATTTCAGTCA GTTGCTCAAT GTACCTATAA CCAGACCGTT CAGCTGGATA
781 TTACGGCCTT TTTAAAGACC GTAAAGAAAA ATAAGCACAA GTTTTATCCG GCCTTTATTC
841 ACATTCTTGC CCGCCTGATG AATGCTCATC CGGAATTCGG TATGGCAATG AAAGACGGTG
901 AGCTGGTGAT ATGGGATAGT GTTACCCTTT GTTACACCGT TTTCCATGAG CAAACTGAAA
961 CGTTTTTCATC GCTCTGGAGT GAATACCACG ACGATTTCCG GCAGTTTCTA CACATATATT
1021 CGCAAGATGT GCGGTGTTAC GGTGAAAACC TGGCCTATTT CCCTAAAGGG TTTATTGAGA
1081 ATATGTTTTT CGTCTCAGCC AATCCCTGGG TGAGTTTCAC CAGTTTTGAT TTAAACGTGG
1141 CCAATATGGA CAACTTCTTC GCCCCGCTT TCACCATGGG CAAATATTAT ACGCAAGGCG
1201 ACAAGGTGCT GATGCCGCTG GCGATTGAGG TTCATCATGC CGTCTGTGAT GGCTTCCATG
1261 TCGGCAGAAT GCTTAATGAA TTACAACAGT ACTGCGATGA GTGGCAGGGC GGGGCGTAAT
1321 CTAGAGGATC CGGCTTACTA AAAGCCAGAT AACAGTATGC GTATTTGCGC GCTGATTTTT
1381 GCGGTATAAG AATATATACT GATATGTATA CCCGAAGTAT GTCAAAAAGA GGTGTGCTAT
1441 GAAGCAGCGT ATTACAGTGA CAGTTGACAG CGACAGCTAT CAGTTGCTCA AGGCATATAT
1501 GATGTCAATA TCTCCGGTCT GGTAAGCACA ACCATGCAGA ATGAAGCCCG TCGTCTGCGT
1561 GCCGAACGCT GGAAAGCGGA AAATCAGGAA GGGATGGCTG AGGTCGCCCG GTTTATTGAA
1621 ATGAACGGCT CTTTGTCTGA CGAGAACAGG GACTGGTGAA ATGCAGTTTA AGGTTTACAC
1681 CTATAAAAGA GAGAGCCGTT ATCGTCTGTT TGTGGATGTA CAGAGTGATA TTATTGACAC
1741 GCGCGGGCGA CGGATGGTGA TCCCCCTGGC CAGTGCACGT CTGCTGTCTG ATAAAGTCTC
1801 CCGTGAACCT TACCCGGTGG TGCATATCGG GGATGAAAGC TGGCGCATGA TGACCACCGA
1861 TATGGCCAGT GTGCCGGTCT CCGTTATCGG GGAAGAAGTG GCTGATCTCA GCCACCGCGA
1921 AAATGACATC AAAAACGCCA TTAACCTGAT GTTCTGGGGA ATATAAATGT CAGGCTCCGT
1981 TATACACAGC CAGTCTGCAG GTCGACCATA GTGACTGGAT ATGTTGTGTT TTACAGTATT
2041 ATGTAGTCTG TTTTATATGC AAAATCTAAT TTAATATATT GATATTTATA TCATTTTACG
2101 TTTCTCGTTC AGCTTTCTTG TACAAAGTGG TTCGATATCG GTACCGAGCT CGAATTCAC
2161 GGCCGTCGTT TTACAACGTC GTGACTGGGA AAACCCTGGC GTTACCCAAC TTAATCGCCT
2221 TGCAGCACAT CCCCCTTTCG CCAGCTGGCG TAATAGCGAA GAGGCCCGCA CCGATCGCCC
2281 TTCCCAACAG TTGCGCAGCC TGAATGGCGA ATGGCGCCTG ATGCGGTATT TTCTCCTTAC
2341 GCATCTGTGC GGTATTTTCA ACCGCATATG GTGCACTCTC AGTACAATCT GCTCTGATGC
2401 CGCATAGTTA AGCCAGCCCC GACACCCGCT AACACCCGCT GACGCGCCTT GACGGGCTTG
2461 TCTGCTCCCG GCATCCGCTT ACAGACAAGC TGTGACCGTC TCCGGGAGCT GCATGTGTCA
2521 GAGGTTTTCA CCGTCATCAC CGAAACGCGC GAGACGAAAG GGCCTCGTGA TACGCCTATT
2581 TTTATAGGTT AATGTCATGA TAATAATGGT TTCTTAGACG TCAGGTGGCA CTTTTCGGGG
2641 AAATGTGCGC GGAACCCCTA TTTGTTTATT TTTCTAAATA CATTCAAATA TGTATCCGCT
2701 CATGAGACAA TAACCTGAT AAATGCTTCA ATAATATTGA AAAAGGAAGA GTATGAGTAT
2761 TCAACATTTT CGTGTGCCCC TTATTCCTT TTTTGCAGCA TTTTGCCTTC CTGTTTTTGC-

```

Figure 83B

2821 TCACCCAGAA ACGCTGGTGA AAGTAAAAGA TGCTGAAGAT CAGTTGGGTG CACGAGTGGG  
2881 TTACATCGAA CTGGATCTCA ACAGCGGTAA GATCCTTGAG AGTTTTCGCC CCGAAGAACG  
2941 TTTTCCAATG ATGAGCACTT TTAAAGTTCT GCTATGTGGC GCGGTATTAT CCCGTATTGA  
3001 CGCCGGGCAA GAGCAACTCG GTCGCCGCAT ACACTATTCT CAGAATGACT TGGTTGAGTA  
3061 CTCACCAAGT ACAGAAAAGC ATCTTACGGA TGGCATGACA GTAAGAGAAT TATGCAGTGC  
3121 TGCCATAACC ATGAGTGATA ACACTCGGC CAACTTACTT CTGACAACGA TCGGAGGACC  
3181 GAAGGAGCTA ACCGCTTTTT TGCACAACAT GGGGGATCAT GTAACTCGCC TTGATCGTTG  
3241 GGAACCGGAG CTGAATGAAG CCATACCAAA CGACGAGCGT GACACCACGA TGCCTGTAGC  
3301 AATGGCAACA ACGTTGCGCA AACTATTAAC TGGCGAACTA CTTACTCTAG CTTCCCGGCA  
3361 ACAATTAATA GACTGGATGG AGGCGGATAA AGTTGCAGGA CCACTTCTGC GCTCGGCCCT  
3421 TCCGGCTGGC TGGTTTATTG CTGATAAATC TGGAGCCGGT GAGCGTGGGT CTCGCGGTAT  
3481 CATTGCAGCA CTGGGGCCAG ATGGTAAGCC CTCCCGTATC GTAGTTATCT ACACGACGGG  
3541 GAGTCAGGCA ACTATGGATG AACGAAATAG ACAGATCGCT GAGATAGGTG CCTCACTGAT  
3601 TAAGCATTGG TAACTGTCAG ACCAAGTTTA CTCATATATA CTTTAGATTG ATTTAAAAC  
3661 TCATTTTAA TTTAAAAGGA TCTAGGTGAA GATCCTTTTT GATAATCTCA TGACCAAAT  
3721 CCCTTAACGT GAGTTTTCGT TCCACTGAGC GTCAGACCCC GTAGAAAAGA TCAAAGGATC  
3781 TTCTTGAGAT CCTTTTTTTC TGCGCGTAAT CTGCTGCTTG CAAACAAAAA AACCACCGCT  
3841 ACCAGCGGTG GTTTGTTTGC CGGATCAAGA GCTACCAACT CTTTTCCGA AGGTAAGTGG  
3901 CTTCAGCAGA GCGCAGATAC CAAATACTGT CCTTCTAGTG TAGCCGTAGT TAGGCCACCA  
3961 CTTCAAGAAC TCTGTAGCAC CGCCTACATA CCTCGCTCTG CTAATCCTGT TACCAGTGGC  
4021 TGCTGCCAGT GGCATAAGT CGTGTCTTAC CGGGTTGGAC TCAAGACGAT AGTTACCGGA  
4081 TAAGGCGCAG CGGTGCGGCT GAACGGGGGG TTCGTGCACA CAGCCAGCT TGGAGCGAAC  
4141 GACCTACACC GAACTGAGAT ACCTACAGCG TGAGCTATGA GAAAGCGCCA CGCTTCCCGA  
4201 AGGGAGAAAG GCGGACAGGT ATCCGGTAAG CGGCAGGGTC GGAACAGGAG AGCGCACGAG  
4261 GGAGCTTCCA GGGGAAACG CCTGGTATCT TTATAGTCTT GTCGGGTTTC GCCACCTCTG  
4321 ACTTGAGCGT CGATTTTTGT GATGCTCGTC AGGGGGGCGG AGCCTATGGA AAAACGCCAG  
4381 CAACGCGGCC TTTTACGGT TCCTGGCCTT TTGCTGGCCT TTGCTCACA TGTTCTTCC  
4441 TGCCTTATCC CCTGATTCTG TGGATAACCG TATTACCGCC TTTGAGTGAG CTGATACCGC  
4501 TCGCCGCAGC CGAACGACCG AGCGCAGCGA GTCAGTGAGC GAGGAAGCGG AAGA



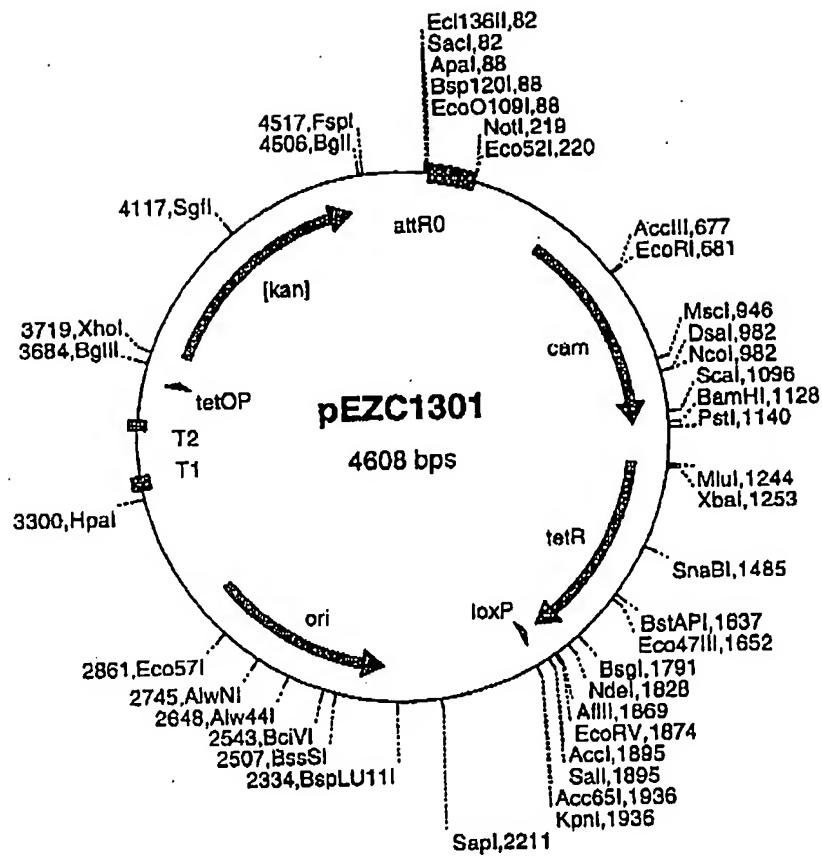


FIGURE 84

196/240

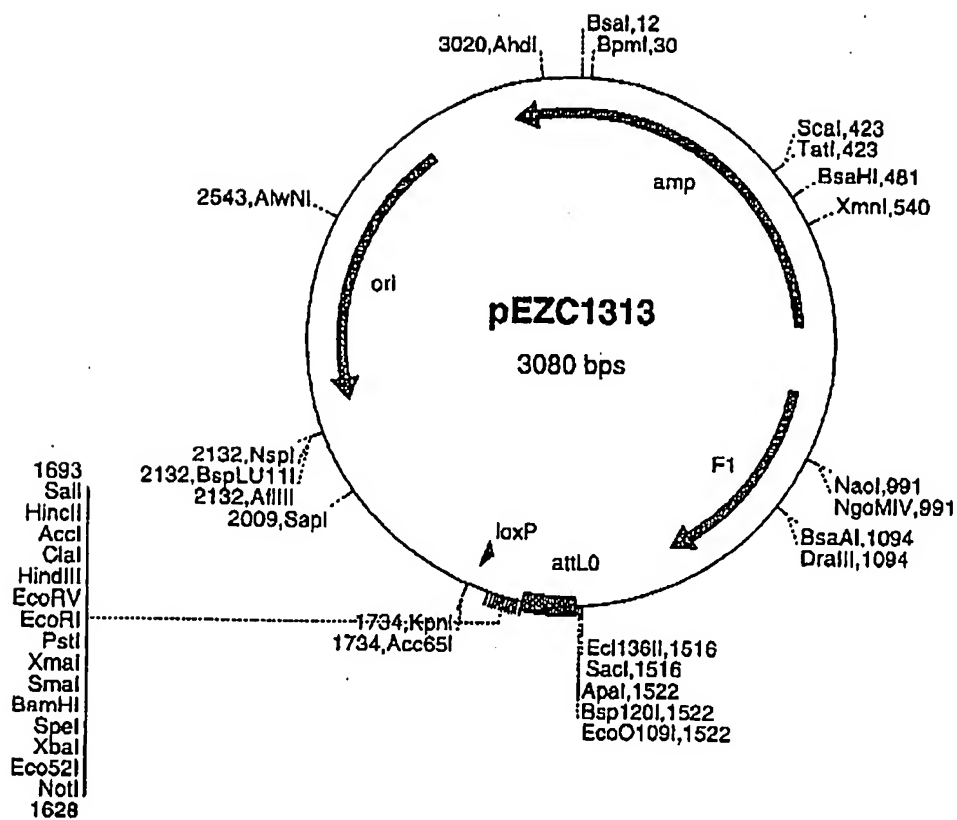


FIGURE 85

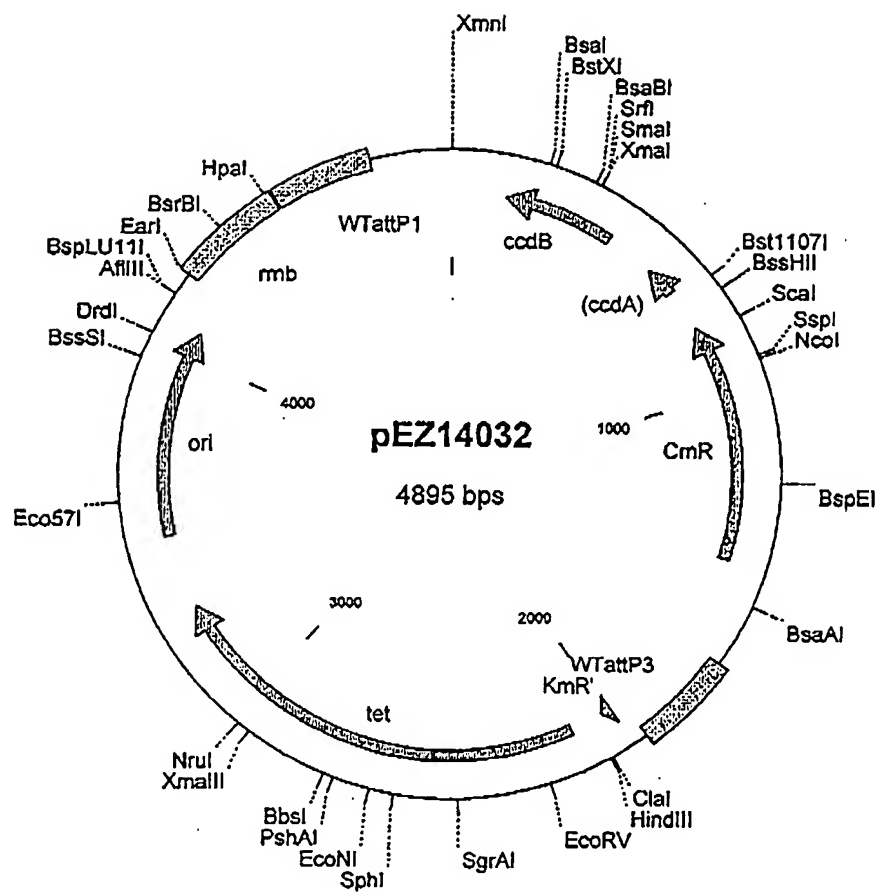


FIGURE 86

198/240

FIGURE 87

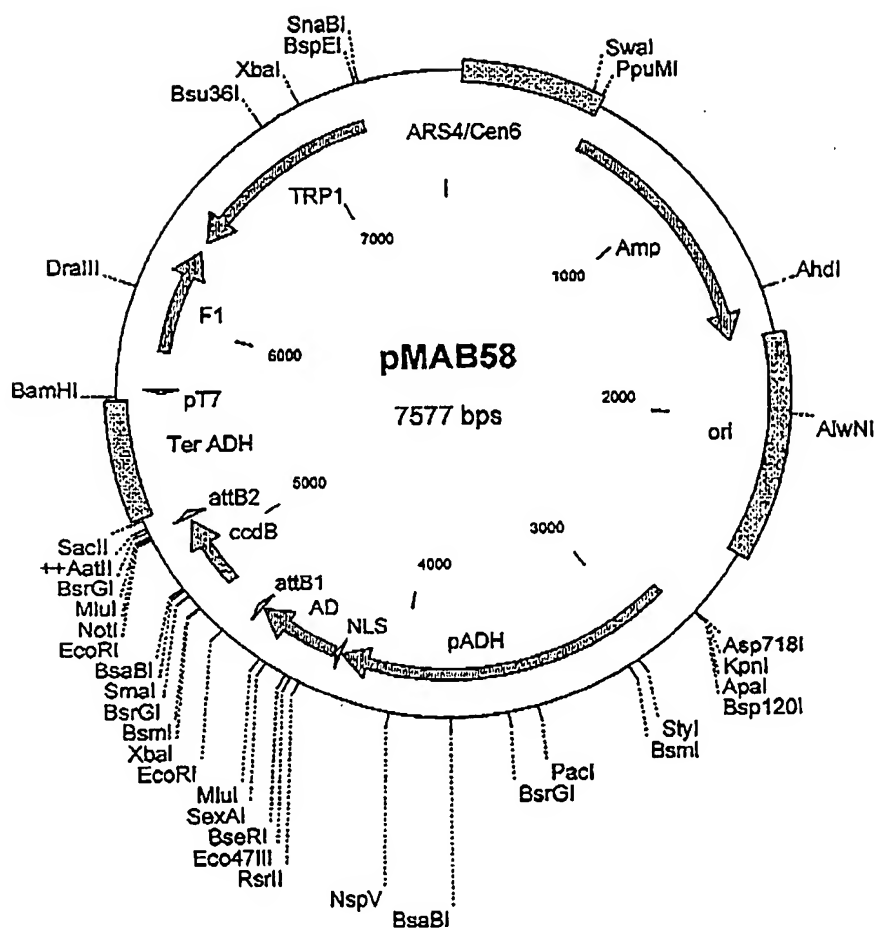
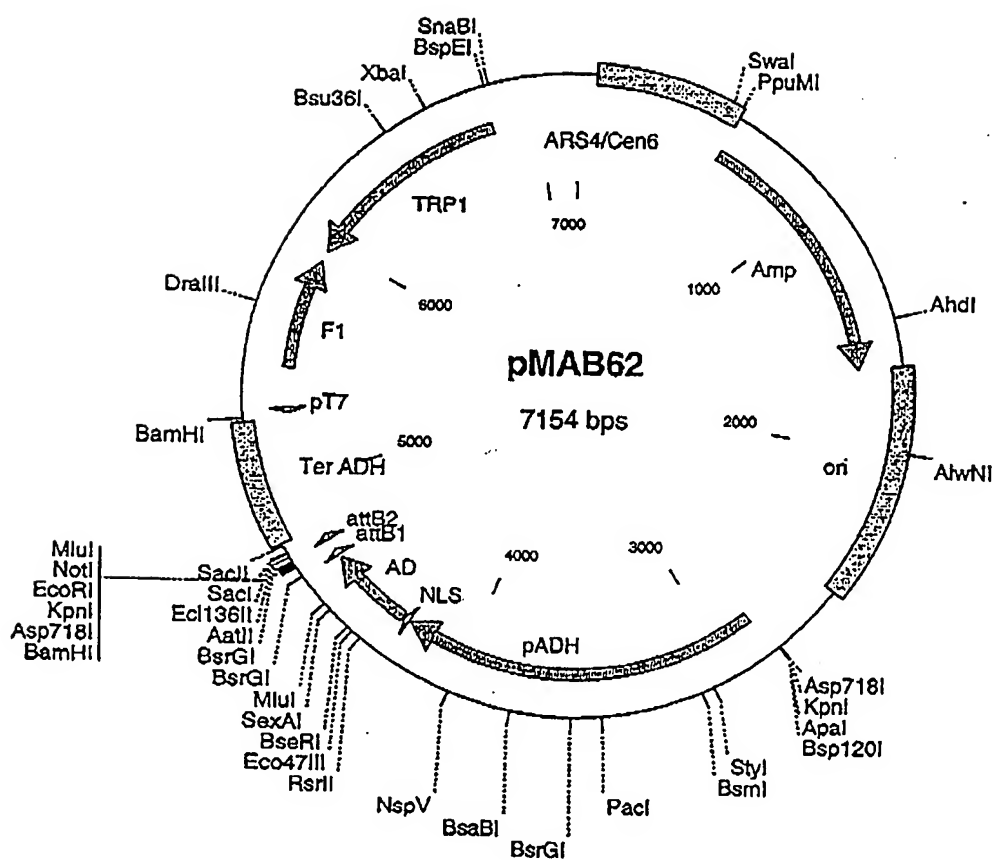
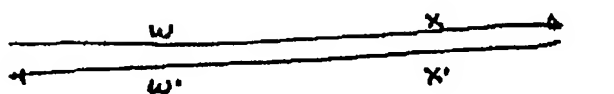


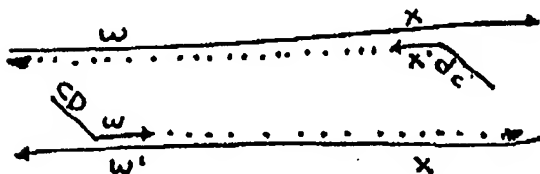
FIGURE 88



DNA to be amplified (5' → 3'):



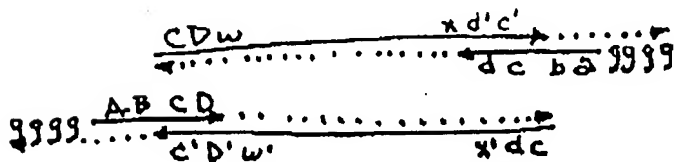
↓ Denature, anneal  
hybrid primers,  
↓ extend with polymerase



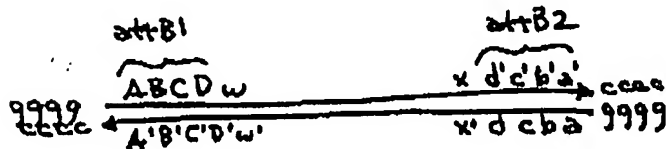
↓ amplification cycles



↓ Denature, anneal  
attB primers,  
extend with polymerase



↓ amplification cycles



attB1 primer:

9999 ABCD →

attB2 primer:

9999 abcd →

Hybrid primers (part  
attB, part gene  
specific):

c d w

e d x'

FIGURE 89

201/240

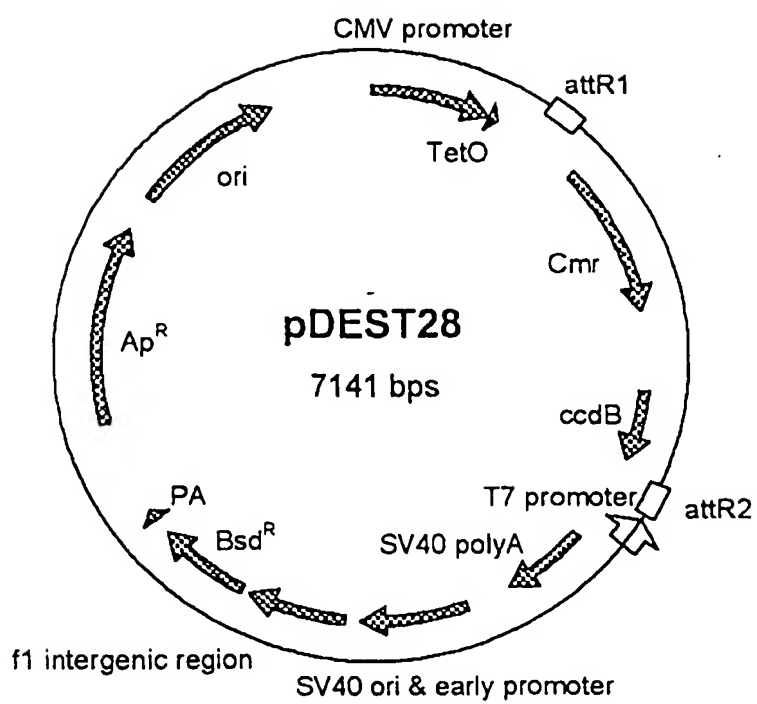


FIGURE 90A

pDEST28 7141 bp

ATGCATGTCGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCC  
CGCCCATTTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCAT  
TGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGAT  
CATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTAT  
GCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATC  
GCTATTACCATGGTGATGCGGTTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTTGAC  
TCACGGGGATTTCCAAGTCTCCACCCATTGACGTCAATGGGAGTTTTGTTTTGGCACCAA  
AATCAACGGGACTTTCAAAATGTGTAACAACCTCCGCCCATTTGACGCAAATGGGCGGT  
AGGCGTGACGGTGGGAGGTCTATATAAGCAGAGCTCTCCCTATCAGTGATAGAGATCTC  
CCTATCAGTGATAGAGATCGTCGACGAGCTCGTTTGTGAACCGTCAGATCGCCTGGAGA  
CGCCATCCACGCTGTTTTGACCTCCATAGAACACCGGGACCGATCCAGCCTCCGGACT  
CTAGAGTACCTACCGGTGATATCCTCGAGCCCATCAACAAGTTTGTACAAAAAGCTG  
AACGAGAAACGTAAATGATATAAATATCAATATATTAATTTAGATTTTGCATAAAAAAC  
AGACTACATAAATACTGTAAAAACACAATATCCAGTCACTATGGCGGCCGCTTAGGCAC  
CCCAGGCTTTACACTTTATGCTTCCGGCTCGTATAATGTGTGGATTTTGTAGTTAGGATCC  
GGCGAGATTTTCAGGAGCTAAGGAAGCTAAATGGAGAAAAAATCACTGGATATACCAC  
CGTTGATATATCCCAATGGCATCGTAAAGAACATTTTGTAGGCATTTTGTAGTGTGCTCA  
ATGTACCTATAACGACCGCTTTCAGCTGGATATTACGGCCTTTTTTAAAGACCGTAAAGAA  
AAATAAGCACAAGTTTTATCCGGCTTTTATTCACATTCTTGCCCGCTGATGAATGCTCA  
TCCGGAATTCCGTATGGCAATGAAAGACGGTGAGCTGGTGATATGGGATAGTGTTTACCCC  
TTGTTACACCGTTTTTCCATGAGCAAACTGAAACGTTTTTTCATCGCTCTGGAGTGAATACCA  
CGACGATTTCCGGCAGTTTCTACACATATATTCGCAAGATGTGGCGTGTTACGGTGAAGAA  
CCTGGCCTATTTCCCTAAAGGGTTTATTTGAGAATATGTTTTTTCGTCTCAGCCAATCCCTG  
GGTGAGTTTTCACCAGTTTTGATTTAAACGTGGCCAATATGGACAACCTTCTTCGCCCCCGT  
TTTTACCATGGGCAAAATATTATACGCAAGGCGACAAGGTGCTGATGCCGCTGGCGATCA  
GGTTTCATCATGCCGTCTGTGATGGCTTCCATGTGCGCAGAATGCTTAATGAATTACAACA  
GTACTGCGATGAGTGGCAGGGCGGGGCGTAAAGATCTGGATCCGGCTTACTAAAAGCCAG  
ATAACAGTATGCGTATTTGCGCGCTGATTTTTTGGCGTATAAGAATATATACTGATATGTA  
TACCCGAAGTATGTCAAAAAGAGGTGTGCTATGAAGCAGCGTATTACAGTGACAGTTGAC  
AGCGACAGCTATCAGTTGCTCAAGGCATATATGATGTCAATATCTCCGGTCTGGTAAGCA  
CAACCATGCAGAAATGAAGCCCGTCTGCTGCGTGCCGAACGCTGGAAAGCGGAAATCAGG  
AAGGGATGGCTGAGGTGCGCCGCTTTATTGAAATGAACGGCTCTTTTGTGACGAGAACA  
GGGACTGGTGAAATGCAGTTTAAAGTTTACACCTATAAAAAGAGAGAGCCGTTATCGTCTG  
TTTGTGGATGTACAGAGTGATATTATTGACACGCCCCGGGCGACGGATGGTGATCCCCCTG  
GCCAGTGCACGTCTGCTGTGATGATAAAGTCTCCCGTGAACCTTTACCCGGTGGTGATATC  
GGGGATGAAAGCTGGCGCATGATGACCACCGATATGGCCAGTGTGCCGGTCTCCGTTATC  
GGGGAAGAAGTGGCTGATCTCAGCCACCGCGAAATGACATCAAAAACGCCATTAACTG  
ATGTTCTGGGGAATATAAATGTGAGGCTCCCTTATACACAGCCAGTCTGCAGGTCGACCA  
TAGTGACTGGATATGTTGTGTTTTACAGTATTATGTAGTCTGTTTTTTATGCAAAATCTA  
ATTTAATATATTGATATTTATATCATTTTTACGTTTCTCGTTTCTGTTTGTACAAAGT  
GGTTGATGGGCGGCCGCTCTAGAGGGCCCAAGCTTACGCGTGCATGCGACGTCATAGCTC  
TCTCCCTATAGTGAGTCGTATTATAAGCTAGGCACTGGCCGTGTTTTTACAACGTCGTGA  
CTGGGAAAACCTGCTAGCTTGGGATCTTTGTGAAGGAACCTTACTTCTGTGGTGTGACATA  
ATTGGACAAACTACCTACAGAGATTTAAAGCTCTAAGGTAAATATAAAATTTTTAAGTGT  
ATAATGTGTTAAACTAGCTGCATATGCTTGTGCTTGTGAGAGTTTGTCTTACTGAGTATGA  
TTTATGAAAATATTATACACAGGAGCTAGTGATTCTAATTGTTTGTGTTTTTTAGATTCA  
CAGTCCCAAGGCTCATTTTCAAGCCCCCTCAGTCTCACAGTCTGTTTATGATCATAATCAG  
CCATACCACATTTGTAGAGGTTTTTACTTGCTTTAAAAAACCTCCACACCTCCCCCTGAA  
CCTGAAACATAAAATGAATGCAATTGTTGTTGTTAACTTGTATTGTCAGCTTATAATGG  
TTACAAATAAAGCAATAGCATCACAATTTTCAAAATAAAGCATTTTTTTTCACTGCATTC  
TAGTTGTGGTTTTGTCCAACTCATCAATGTATCTTATCATGTCTGGATCGATCCTGCTGAT  
AATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGGCGTATTGGCTGGCGTAATAGCGAAG  
AGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGGACGCGC  
CCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACAC  
TTGCCAGCGCCCTAGCGCCCGCTCCTTTTCTGCTTTCTTCCCTTCTTCTCGCCACGTTCC  
CCGGCTTTCCCGCTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTT-

Figure 90B



TACGGCACCTCGACCCCAAAAACTTGATTAGGGTGATGGTTACGCTAGTGGGCCATCGC  
CCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCT  
TGTTCCAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTTTTGATTATAAGGGA  
TTTTGCCGATTTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAATATTTAACGCGA  
ATTTTAACAAATATTAACGTTTACAATTTTCGCCTGATGCGGTATTTTCTCCTTACGCAT  
CTGTGCGGTATTTTACACCCGCATACGCGGATCTGCGCAGCACCATGGCCTGAAATAACCT  
CTGAAAGAGGAACTTGTTAGGTACCTTCTGAGGCGGAAAGAACCAGCTGTGGAATGTGT  
GTCAGTTAGGGTGTGGAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGC  
ATCTCAATTAGTCAGCAACCAGGTGTGGAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTA  
TGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCC  
CGCCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTTTTTTTA  
TTTATGCAGAGGCCGAGGCCCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCT  
TTTTTGAGGCCCTAGGCTTTTGCAAAAAGCTTGATTCTTCTGACACAACAGTCTCGAAT  
TGAACCATGGCCAAGCCTTTGTCTCAAGAAGAATCCACCCTCATTGAAAGAGCAACGGC  
TACAATCAACAGCATCCCCATCTCTGAAGACTACAGCGTCGCCAGCGCAGCTCTCTCTAG  
CGACGGCCGCATCTTCACTGGTGTCAATGTATATCATTTTTACTGGGGACCTTGTGCAGA  
ACTCGTGGTGCTGGGCACTGCTGCTGCTGCGGCAGCTGGCAACCTGACTTGTATCGTCGC  
GATCGGAAATGAGAACAGGGGCATCTTGAGCCCCTGCGGACGGTGCCGACAGGTGCTTCT  
CGATCTGCATCCTGGGATCAAAGCCATAGTGAAGGACAGTGATGGACAGCCGACGGCAGT  
TGGGATTCGTGAATTGCTGCCCTCTGGTTATGTGTGGGAGGGCTAAGCACTTCGTGGCCG  
AGTTCGAAATGACCGACCAAGCGACGCCCAACCTGCCATCACGATGGCCGCAATAAAATA  
TCTTTATTTTTATTACATCTGTGTGTGGTTTTTTGTGTGAATCGATAGCGATAAGGATC  
CGCGTATGGTGCACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGCCCCGA  
CACCCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTAC  
AGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTGAGAGTTTTACCGTCATCACCG  
AAACGCGCGAGACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTCAATGATA  
ATAATGGTTTTCTTAGACGTGAGGTGGCATTCTTTCGGGGAAATGTGCGCGGAACCCCTATT  
TGTTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAA  
ATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTGCCCTT  
ATTCCCTTTTTTTCGGCATTTTTGCCTTCCTGTTTTTGTCTCACCCAGAAACGCTGGTGAAA  
GTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAAC  
AGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAAGCTTTTCCAATGATGAGCACTTTT  
AAAGTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGT  
CGCCGCATACATATTCTCAGAATGACTTGTTGAGTACTCACCAGTCACAGAAAAGCAT  
CTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAAC  
ACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTG  
CACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCC  
ATACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAA  
CTATTAACCTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAG  
GCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTTATTGCT  
GATAAATCTGGAGCCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGAT  
GGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAA  
CGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACGTGACAG  
CAAGTTTACTCATATATACTTTAGATTGATTTAAACTTCATTTTTTAATTTAAAGGATC  
TAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTC  
CACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTG  
CGCGTAATCTGCTGCTTGCAAAACAAAAAACACCGCTACCAGCGGTGGTTTTGTTGCCG  
GATCAAGAGCTACCAACTCTTTTTCCGAAGGTAAGTGGCTTCAGCAGAGCGCAGATACCA  
AATACTGTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCG  
CCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCCG  
TGTCTTACCGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCCGGCTGA  
ACGGGGGTTTCGTGCACACAGCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATAC  
CTCAGACGCTGAGCATTGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTAT  
CCGTAAGAGCGGAGGTCGGAACAGGAGCGCACGAGGGAGCTTCCAGGGGAAACGCC  
TGGTATCTTTATAGTCTGTGCGGTTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGA  
TGCTCGTCAGGGGGCGGAGCCTATGGAACACGCCAGCAACGCGGCCTTTTTACGGTTC  
CTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCTGATTCTGTG  
GATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGACGCCGAACGACCGAG-

204/240

CGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCC  
GCGCGTTGGCCGATTCATTAATGCAGAGCTTGCAATTCGCGCGTTTTTCAATATTATTGA  
AGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAAT  
AAACAAATAGGGTTCCGCGCACATTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACC  
ATTATTATCATGACATTAACCTATAAAAAATAGGCGTAGTACGAGGCCCTTCACTCATT  
G

FIGURE 90D

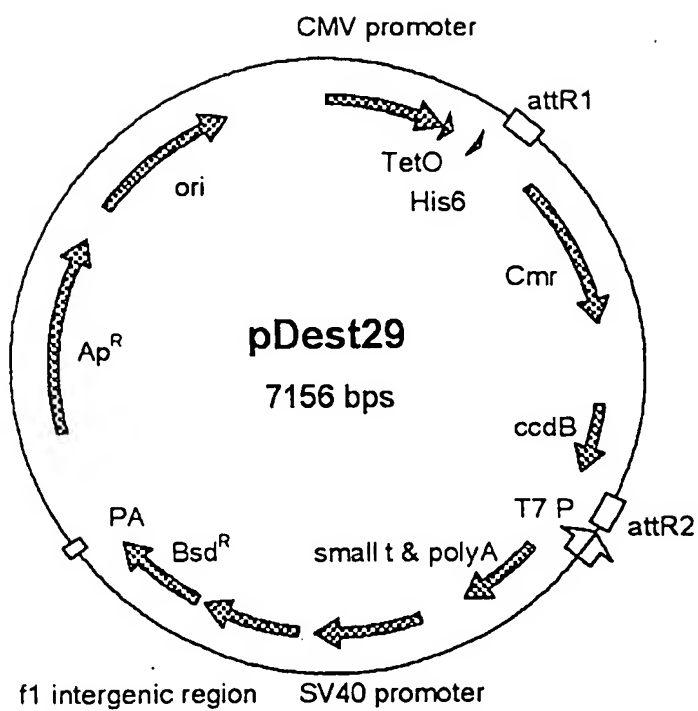


FIGURE 91 A

pDEST29 7156 bp

ATGCATGTCGTTACATAACTTACGGTAAATGGCCCCGCTGGCTGACCGCCCAACGACCCC  
CGCCCATTTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCAT  
TGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCACTTGGCAGTACATCAAGTGTAT  
CATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCCGCTGGCATTAT  
GCCCAGTACATGACCTTATGGGACTTTCTACTTGGCAGTACATCTACGTATTAGTCATC  
GCTATTACCATGGTGTATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTTGAC  
TCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAA  
AATCAACGGGACTTTCCAAAATGTCGTAACAACCTCCGCCCCATTGACGCAAATGGGCGGT  
AGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCTCCCTATCAGTGATAGAGATCTC  
CCTATCAGTGATAGAGATCGTCGACGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGA  
CGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCCAGCCTCCGGACC  
ATGGCGTACTACCATCACCATCACCATCACACCGGTGATATCCTCGAGCCCATCACAAGT  
TTGTACAAAAAAGCTGAACGAGAAACGTAAAATGATATAAATATCAATATATTAAATTAG  
ATTTTGCATAAAAAACAGACTACATAATACTGTAAACACAAATATCCAGTCACTATGG  
CGGCCGATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATAATGTGTGGA  
TTTTGAGTTAGGATCCGGCGAGATTTTCAGGAGCTAAGGAAGCTAAAATGGAGAAAAAAA  
TCTATGGATATACCAACCGTTGATATATCCCAATGGCATCGTAAAGAACATTTTGGGAGA  
TTCAGTCAGTTGCTCAATGTACCTATAACCAGACCGTTTCAGCTGGATATTACGGCCTTTT  
TAAAGACCGTAAAGAAAAATAAGCACAAGTTTTATCCGGCCTTTATTACATTCTTGCCC  
GCCTGATGAATGCTCATCCGGAATTCGCTATGGCAATGAAAGACGGTGAGCTGGTGATAT  
GGGATAGTGTTTACCCCTTGTTACACCGTTTTCCATGAGCAAACTGAAACGTTTTTCATCGC  
TCTGGAGTGAATACCACGACGATTTCCGGCAGTTTCTACACATATATTGCAAGATGTGG  
CGTGTTACGGTGAAAACCTGGCCTATTTCCCTAAAGGGTTTATTGAGAATATGTTTTTCG  
TCTCAGCCAATCCCTGGGTGAGTTTCCACGATTTTGATTAAACGTTGGCCAATATGGACA  
ACTTCTTCGCCCCCGTTTTCCACCATGGGCAAATATTATACGCAAGGCGACAAGGTGCTGA  
TGCCGCTGGCGATTTCAGGTTTCATCATGCCGTCTGTGATGGCTTCCATGTCCGCAGAATGC  
TTAATGAATTACAACAGTACTGCGATGAGTGGCAGGGCGGGCGTAAACGCGTGGATCCG  
GCTTACTAAAAGCCAGATAACAGTATGCGTATTTGCGCGCTGATTTTTGCGGTATAAGAA  
TATATACTGATATGTATACCCGAAGTATGTCAAAAAGAGGTGTGCTATGAAGCAGCGTAT  
TACAGTGACAGTTGACAGCGACAGCTATCAGTTGCTCAAGGCATATATGATGTCAATATC  
TCCGGTCTGGTAAAGCACAACCATGCAGAATGAAGCCCGTCTGCTGCGTGCCGAACGCTGG  
AAAGCGGAAAATCAGGAAGGGATGGCTGAGGTGCGCCCGTTTTATTGAAATGAACGGCTCT  
TTTGCTGACGAGAACAGGGACTGGTGAAATGCAGTTTAAGGTTTACACCTATAAAAGAGA  
GAGCCGTTATCGTCTGTTTGTGGATGTACAGAGTGATATTATTGACACGCCCGGGCGACG  
GATGGTGATCCCCCTGGCCAGTGACGCTGCTGTGTCAGATAAAGTCTCCCGTGAACTTTA  
CCCCGTGGTGATATCGGGGATGAAAGCTGGCGCATGATGACCACCGATATGGCCAGTGT  
GCCGTCTCCGTTATCGGGGAAGAAGTGGCTGATCTCAGCCACCGCGAAAATGACATCAA  
AAACGCCATTAAACCTGATGTTTCTGGGGAATATAAATGTACAGGCTCCGTTATACACAGCCA  
GTCTGCAGGTGACCATAGTGACTGGATATGTTGTGTTTTACAGTATTATGTAGTCTGTT  
TTTTATGCAAAATCTAATTTAATATATTGATATTTATATCATTTTTACGTTTCTCGTTCAG  
CTTTCTGTACAAAGTGGTGATGGGCGGCCGCTCTAGAGGGCCCAAGCTTACGCGTGAT  
GCGACGTCATAGCTCTCTCCCTATAGTGAGTCGTATTATAAGCTAGGCACTGGCCGTCGT  
TTTACAACGTCGTGACTGGGAAAACCTGCTAGCTTGGGATCTTTGTGAAGGAACCTTACTT  
CTGTGGTGTGACATAATTGGACAAACTACCTACAGAGATTTAAAGCTCTAAGGTAATAT  
AAAATTTTTAAGTGTATAATGTGTTAAACTAGCTGCATATGCTTGCTGCTTGAGAGTTTT  
GCTTACTGAGTATGATTTATGAAAATATTATACACAGGAGCTAGTGATTCTAATTGTTTG  
TGTATTTTAGATTTCACAGTCCCAAGGCTCATTTACAGCCCCCTCAGTCCTCACAGTCTGTT  
CATGATCATAATCAGCCATACCACATTTGTAGAGGTTTTACTTGCTTTAAAAAACCTCCC  
ACACCTCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTTGTTGTTAACTTGTTTAT  
TGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTACAAATAAAGCATT  
TTTTTCACTGATTTCTAGTTGTGGTTTTGTCCAAACTCATCAATGTATCTTATCATGTCTG  
GATCGATCCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTTGCGTATTGGCT  
GGCGTAATAGCGAAGAGGCCCCGACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATG  
GCGAATGGGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCA  
GCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTTCGCTTTCTTCCCTTCCT  
TTCTCGCCACGTTCCGCGGCTTTCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGT-

FIGURE 91B

TCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAACTTGATTAGGGTGATGGTTCAC  
GTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCT  
TTAATAGTGGACTCTTGTTCCAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTT  
TTGATTTATAAGGGATTTTGCCGATTTGGCCCTATTGGTTAAAAAATGAGCTGATTAAAC  
AAATATTTAACGCGAATTTTAACAAAATATTAACGTTTACAATTTTCGCCTGATGCGGTAT  
TTTCTCCTTACGCATCTGTGCGGTATTTACACCCGCATACGCGGATCTGCGCAGCACCAT  
GGCCTGAAAATAACCTCTGAAAGAGGAACCTTGGTTAGGTACCTTCTGAGGCGGAAAGAACC  
AGCTGTGGAATGTGTGTGTCAGTTAGGGTGTGGAAGTCCCCAGGCTCCCCAGCAGGCAGAA  
GTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAGTCCCCAGGCTCCC  
CAGCAGGCAGAAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCCGCCCC  
TAACTCCGCCCCATCCGCCCCCTAACTCCGCCCCAGTTCCGCCCCATTCTCCGCCCCATGGCT  
GACTAATTTTTTTTATTTATGTCAGAGGCCGAGGCCGCTCGGCCTCTGAGCTATTCCAGA  
AGTAGTGGAGGGCTTTTTTGGAGGCCATGAGCTTTTGCAAAAAGCTTGATTCTTCTGACA  
CAACAGTCTCGAACTTAAGACCATGGCCAAGCCTTTGTCTCAAGAAGAATCCACCCTCAT  
TGAAAGAGCAACGGCTACAATCAACAGCATCCCCATCTCTGAAGACTACAGCGTCGCCAG  
CGCAGCTCTCTCTAGCGACGGCCGCATCTTCACTGGTGTCAATGTATATCATTTTACTGG  
GGGACCTTGTGCGAAGTCTGTGGTGTGGGCACTGCTGCTGCTGCGGCAGCTGGCAACCT  
GACTTGTATCGTCGCGATCGGAAATGAGAACAGGGGCATCTTGAGCCCCCTGCGGACGGTG  
CCGACAGGTGCTTCTCGATCTGCATCCTGGGATCAAGCCATAGTGAAGGACAGTGATGG  
ACAGCCGACGGCAGTTGGGATTCGTGAATTGCTGCCCTCTGGTTATGTGTGGGAGGGCTA  
AGCACTTCGTGGCCGAGTTCGAAATGACCGACCAAGCGACGCCCAACCTGCCATCACGAT  
GGCCGCAATAAAATATCTTTATTTTATTACATCTGTGTGTTGGTTTTTTGTGTGAATCG  
ATAGCGATAAGGATCCGCGTATGGTGCATCTCAGTACAATCTGCTCTGATGCCGCATAG  
TTAAGCCAGCCCCGACACCCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTC  
CCGGCATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTGAGAGGTTT  
TCACCGTCATCACCGAAACGCGGAGACGAAAGGGCCTCGTGATACGCCATTTTTATAG  
GTTAATGTCTAGATAATAATGGTTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTG  
CGCGGAACCCCTATTTGTTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGA  
CAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACAT  
TTCCGTGTGCGCCCTTATTCCTTTTTTGGCGCATTTTGCCTTCCTGTTTTTGCTCACCCA  
GAAACGCTGGTGAAAGTAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTATCATC  
GAAGTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTTCGCCCCGAAGAACGTTTTCCA  
ATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGG  
CAAGAGCAACTCGGTGCGCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTACCA  
GTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATA  
ACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAG  
CTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACCTCGCCTTGATCGTTGGGAACCG  
GAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCA  
ACAACGTTGCGCAAACATTAAGTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTA  
ATAGACTGGATGGAGGCGGATAAAGTTGACGAGCACTTCTGCGCTCGGCCCTTCCGGCT  
GGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCA  
GCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAG  
GCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCAT  
TGGTAACCTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTT  
TAATTTAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAA  
CGTGAGTTTTCTGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGA  
GATCCTTTTTTTCTGCGCGTAATCTGTGCTTGCAAAACAAAAAACACCGCTACCAGCG  
GTGGTTTTGTTGCGCGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAAGTGGCTTCAGC  
AGAGCGCAGATAACCAATACTGTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAG  
AACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAAGTGGCTGCTGCC  
AGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCG  
CAGCGGTGCGGCTGAACGGGGGGTTCGTGCACACAGCCAGCTTGGAGCGAAGGAGTAC  
ACCGAAGTCTGAGATACCTACAGCGTGAGCATTGAGAAAGCGCCACGCTTCCCGAAGGGAGA  
AAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCCGAACAGGAGAGCGCACGAGGGAGCTT  
CCAGGGGGAAACGCTGGTATCTTTATAGTCTGTGCGGTTTTCGCCACCTCTGACTTGAG  
CGTCGATTTTTGTGATGCTCGTCAGGGGGCGGAGCCTATGAAAAACGCCAGCAACGCG  
GCCTTTTTACGGTTCTTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCTGCGTTA  
TCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGC-

FIGURE 91C

AGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGC  
AAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGAGCTTGCAATTCGCGCGTT  
TTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAA  
TGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCGAAAAGTGCCACCT  
GACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTAGTACGAGG  
CCCTTTCATCATTAG

FIGURE 91D

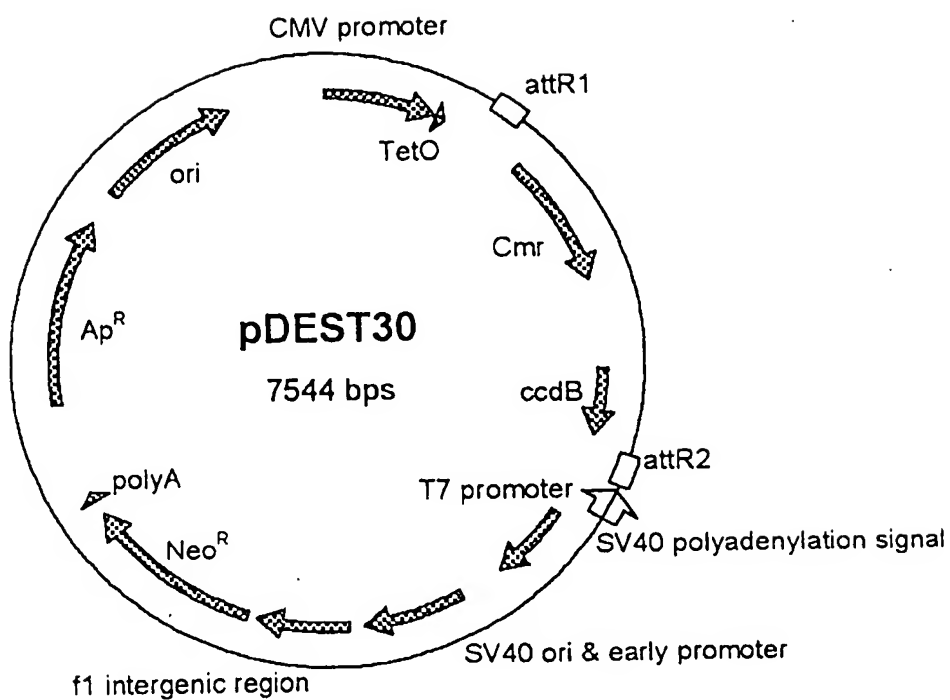


FIGURE 92A

pDEST30

7544 bp

ATGCATGTCGTTACATAA CTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCC  
CGCCCATTTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCAT  
TGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTAT  
CATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTAT  
GCCCAGTACATGACCTTATGGGACTTTCTACTTGGCAGTACATCTACGTATTAGTCATC  
GCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTTGAC  
TCACGGGGATTTC AAGTCTCCACCCCATTTGACGTCAATGGGAGTTTGT TTTGGCACCAA  
AATCAACGGGACTTTCCAAAATGTCTGAACAACTCCGCCCCATTGACGCAAATGGGCGGT  
AGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCTCCCTATCAGTGATAGAGATCTC  
CCTATCAGTGATAGAGATCGTCGACGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGA  
CGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCCAGCCTCCGGACT  
CTAGAGGATCCCTACCGGTGATATCCTCGAGCCCATCAACAAGTTTGTACAAAAAAGCTG  
AACGAGAAACGTAAAAATGATATAAATATCAATATATTAATTAGATTTTGCATAAAAAAC  
AGACTACATAACTGTAAAAACACAACATATCCAGTCACTATGGCGGCCGATTAGGCAC  
CCCAGGCTTTACACTTTATGCTTCCGGCTCGTATAATGTGTGGATTTTGTAGTTAGGATCC  
GGCGAGATTTTCAGGAGCTAAGGAAGCTAAAAATGGAGAAAAAATCACTGGATATACCAC  
CGTTGATATATCCCAATGGCATCGTAAAGAACATTTTGAGGCATTTTCAGTCAGTTGCTCA  
ATGTACCTATAACCAGACCGTTTCAGCTGGATATTACGGCCTTTTTAAAGACCGTAAAGAA  
AAATAAGCACAAGTTTTATCCGGCCTTTATTCACATTCTTGCCCGCTGATGAATGCTCA  
TCCGGAATTCGGTATGGCAATGAAAGACGGTGAGCTGGTGATATGGGATAGTGTTCACCC  
TTGTTACACCGTTTTTCCATGAGCAAACTGAAACGTTTTTCATCGCTCTGGAGTGAATACCA  
CGACGATTTCCGGCAGTTTCTACACATATATTCGCAAGATGTGGCGTGTACGGTGAAAA  
CCTGGCCTATTTCCCTAAAGGGTTTTATTGAGAATATGTTTTTCGTCTCAGCCAATCCCTG  
GGTGAGTTTTACCAGTTTTGATTTAAACGTGGCCAATATGGACAACCTCTTCGCCCCCGT  
TTTCACCATGGGCAAATATTATACGCAAGGCGACAAGGTGCTGATGCCGCTGGCGATTCA  
GGTTCATCATGCCGTCTGTGATGGCTTCCATGTCGGCAGAATGCTTAATGAATTACAACA  
GTACTGCGATGAGTGGCAGGGCGGGCGTAAAGATCTGGATCCGGCTTACTAAAAGCCAG  
ATAACAGTATGCGTATTTCGCGCTGATTTTTGCGGTATAAGAATATATACTGATATGTA  
TACCCGAAGTATGTCAAAAAGAGGTGTGCTATGAAGCAGCGTATTACAGTGACAGTTGAC  
AGCGACAGCTATCAGTTGCTCAAGGCATATATGATGTCAATATCTCCGGTCTGGTAAGCA  
CAACCATGCAGAATGAAGCCCGTCTGCTGCGTGCCGAACGCTGGAAAGCGGAAAATCAGG  
AAGGGATGGCTGAGGTGCGCCCGTTTTATTGAAATGAACGGCTCTTTTGCTGACGAGAAC  
GGGACTGGTGAAATGCAGTTTAAGGTTTACACCTATAAAAGAGAGAGCCGTTATCGTCTG  
TTTGTGGATGTACAGAGTGAATATTATTGACACGCCCCGGGCGACGGATGGTGATCCCCCTG  
GCCAGTGCACGTCTGCTGTGAGATAAAGTCTCCCGTGAACTTTACCCGGTGGTGATATC  
GGGGATGAAAGCTGGCGCATGATGACCACCGATATGGCCAGTGTGCCGGTCTCCGTTATC  
GGGGAAGAAGTGGCTGATCTCAGCCACCGCGAAAATGACATCAAAAACGCCATTAACTG  
ATGTTCTGGGGAATATAAATGTGAGGCTCCCTTATACACAGCCAGTCTGCAGGTGACCA  
TAGTGACTGGATATGTTGTGTTTTACAGTATTATGTAGTCTGTTTTTTATGCAAAATCTA  
ATTTAATATATTGATATTTATATCATTTTACGTTTTCTCGTTTCAGCTTTCTTGTAACAAGT  
GGTTGATGGGCGGCCGCTCTAGAGGGCCCAAGCTTACGCGTGCATGCGACGTCATAGCTC  
TCTCCCTATAGTGAGTCGTATTATAAGCTAGGCACTGGCCGTGTTTTACAACGTCGTGA  
CTGGGAAAACCTGCTAGCTTGGGATCTTTGTGAAGGAACCTTACTTCTGTGGTGTGACATA  
ATTGGACAAACTACCTACAGAGATTTAAAGCTCTAAGGTAAATATAAAATTTTTAAGTGT  
ATAATGTGTTAAACTAGCTGCATATGCTTGCTGCTTGAGAGTTTTGCTTACTGAGTATGA  
TTTATGAAAATATTATACACAGGAGCTAGTGATTCTAATTGTTTGTGTATTTTAGATTCA  
CAGTCCCAAGGCTCATTTTCAGGCCCTCAGTCCTCACAGTCTGTTTCATGATCATAATCAG  
CCATACCACATTTGTAGAGGTTTTACTTGCTTTAAAAAACCTCCACACCTCCCCCTGAA  
CCTGAAAACATAAAATGAATGCAATTGTTGTTGTTAACTTGTTTATGTCAGCTTATAATGG  
TTACAAATAAAGCAATAGCATCACAATTTTCAAAATAAAGCATTTTTTTCACTGCATTC  
TAGTTGTGGTTTTGTCCAAACTCATCAATGTATCTTATCATGTCTGGATCGATCCTGCATT  
AATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGGCTATTGGCTGGCGTAATAGCGAAG  
AGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGGACGCGC  
CCTGTAGCGCGCATTAAGCGCGGCGGTTGTTGTTACGCGCAGCGTGACCGCTACAC  
TTGCCAGCGCCCTAGCGCCGCTCCTTTTCGCTTTCTTCCCTTCTTCTCGCCACGTTCCG  
CCGGCTTTCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCGATTTAGTGCTT-

Figure 92B



211/240

TACGGCACCTCGACCCCAAAAACTTGATTAGGGTGATGGTTACGTAAGTGGGCCATCGC  
CCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCT  
TGTTCCAACTGGAACAACACTCAACCTATCTCGGTCTATTCTTTTGATTATAAGGGA  
TTTTGCCGATTTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAATATTTAACGCGA  
ATTTTAACAAAATATTAACGTTTACAATTTTCGCCTGATGCGGTATTTCTCCTTACGCAT  
CTGTGCGGTATTTACACCCGCATACGCGGATCTGCGCAGCACCATGGCCTGAAATAACCT  
CTGAAAGAGGAACCTTGGTTAGGTACCTTCTGAGGCGGAAAGAACCAGCTGTGGAATGTGT  
GTCAGTTAGGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGC  
ATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTA  
TGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCCTAACTCCGCCCCATCC  
CGCCCCCTAACTCCGCCCCAGTTCCGCCCCATTCTCCGCCCCATGGCTGACTAATTTTTTTTA  
TTTATGCAGAGGCCGAGGCCGCTCGGCCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCT  
TTTTTGAGGCCCTAGGCTTTTGCAAAAAGCTTGATTCTTCTGACACAACAGTCTCGAAT  
TAAGGCTAGAGCCACCATGATTGAACAAGATGGATTGCACGCAGGTCTCCGGCCGCTTG  
GGTGGAGAGGCTATTTCGGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGC  
CGTGTTCGGCTGTGACGCGAGGGGCGCCCGTTCTTTTGTCAAGACCGACCTGTCCGG  
TGCCCTGAATGAACTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGT  
TCCTTGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGAAGTGGCTGCTATTGGG  
CGAAGTGCCGGGGCAGGATCTCCTGTCTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCAT  
CATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATTCGACCA  
CCAAGCGAAACATCGCATCGAGCGAGCAGTACTCGGATGGAAGCCGGTCTTGTGATCA  
GGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAAGTTCGCCAGGCTCAA  
GGCGCGCATGCCCGACGGCGAGGATCTCGTCTGACCCATGGCGATGCCTGCTTGCCGAA  
TATCATGGTGGAAAATGGCCGCTTTTCTGGATTTCATCGACTGTGGCCGGCTGGGTGTGGC  
GGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGCGA  
ATGGGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCTCCCGATTTCGACGCGCATCGC  
CTTCTATCGCCTTCTTGACGAGTTCTTCTGAGCGGGACTCTGGGGTTTCAAATGACCGAC  
CAAGCGACGCCCAACCTGCCATCAGATGGCCGCAATAAAATATCTTTATTTTTCATTACA  
TCTGTGTGTTGGTTTTTTGTGTGAATCGATAGCGATAAGGATCCGCGTATGGTGCATCT  
CAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGCCCCGACACCCGCCAACACCCGC  
TGACGCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAGACAAGCTGTGACCGT  
CTCCGGGAGCTGCATGTGTGAGAGGTTTTACCCGTCTACCCGAAACGCGCGAGACGAAA  
GGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTCATGATAATAATGGTTTTCTAGAC  
GTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTTATTTTCTAAAT  
ACATTCAAATATGTATCCGCTCATGAGACAATAACCCGTGATAAATGCTTCAATAATATTG  
AAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTGCGCCCTTATTCCCTTTTTTGCGGC  
ATTTTGCTTCTGTTTTTGTCTACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGA  
TCAGTTGGGTGCACGAGTGGGTACATCGAAGTGGATCTCAACAGCGGTAAGATCCTTGA  
GAGTTTTCGCCCCGAAGAACGTTTTTCAATGATGAGCACTTTTAAAGTTCTGCTATGTGG  
CGCGGTATTATCCCGTATTGACGCGGGCAAGAGCAACTCGGTGCGCCGCATACACTATTC  
TCAGAAAGACTTGGTTGAGTACTCACCAGTCAAGAAAAGCATCTTACGGATGGCATGAC  
AGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAAAGTGCAGGCAACTTACT  
TCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCA  
TGTAAGTGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCG  
TGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAAGTGGCGAACT  
ACTTACTCTAGCTTCCCGGCAACAATTAAGACTGGATGGAGGCGGATAAAGTTGCAGG  
ACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTTATTGCTGATAAATCTGGAGCCGG  
TGAGCGTGGGTCTCGCGGTATCATTTGACGCACTGGGGCCAGATGGTAAGCCCTCCCGTAT  
CGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGC  
TGAGATAGGTGCCTCACTGATTAAGCATTGGTAAGTGTGACACCAAGTTTACTCATATAT  
ACTTTAGATTGATTTAAACTTCATTTTTTAATTTAAAGGATCTAGGTGAAGATCCTTTT  
TGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTTCGTTCCACTGAGCGTCAGACCC  
CGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTT  
GCAACAAAAAAACCACCGCTACCAGCGGTGGTTTTGTTTGCCGATCAAGAGTACCAAC  
TCTTTTTCCGAAGGTAAGTGGCTTTCAGCAGAGCGCAGATACCAAACTGTCCTTCTAGT  
GTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCT  
GCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCTGTCTTACCGGGTTGGA  
CTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTGCGGCTGAACGGGGGGTTCGTGCAC-

FIGURE 92C

ACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCATTG  
AGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGT  
CGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCC  
TGTCGGGTTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCG  
GAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCC  
TTTTGCTCACATGTTCTTTCCTGCGTTATCCCTGATTCTGTGGATAACCGTATTACCGC  
CTTTGAGTGAGCTGATACCGCTCGCCGCGAGCCGAACGACCGAGCGCAGCGAGTCAGTGAG  
CGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCA  
TTAATGCAGAGCTTGCAATTCGCGCGTTTTTCAATATTATTGAAGCATTATCAGGGTTA  
TTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCC  
GCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATCATGACATT  
AACCTATAAAAAATAGGCGTAGTACGAGGCCCTTTCATCATTAG

FIGURE 92D

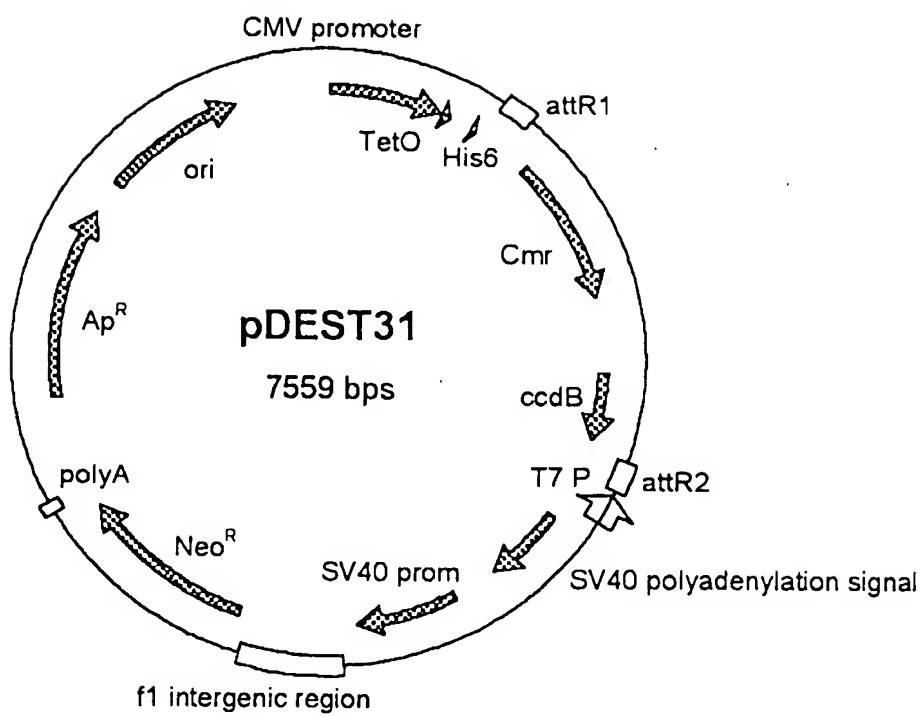


FIGURE 93A

pDEST31 7559 bp

24/240

ATGCATGTCGTTACATAACTTACGGTAAATGGCCCCGCTGGCTGACCGCCCAACGACCCC  
CGCCCATTTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCAT  
TGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTAT  
CATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCCGCTGGCATTAT  
GCCCAGTACATGACCTTATGGGACTTTCTACTTGGCAGTACATCTACGTATTAGTCATC  
GCTATTACCATGGTGATGCGGTTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTTGAC  
TCACGGGGATTTCCTAAGTCTCCACCCATTGACGTCAATGGGAGTTTTGTTTTGGCACCAA  
AATCAACGGGACTTTCCAAAATGTCTGAACAACTCCGCCCCATTGACGCAATGGGCGGT  
AGGCGTGACGGTGGGAGGTCTATATAAGCAGAGCTCTCCCTATCAGTGATAGAGATCTC  
CCTATCAGTGATAGAGATCGTTCGACGAGCTCGTTTTAGTGAACCGTCAGATCGCCTGGAGA  
CGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCCAGCCTCCGGACC  
ATGGCGTACTACCATCACCATCACCATCACACCGGTGATATCCTCGAGCCCATCAAAAGT  
TTGTACAAAAAAGCTGAACGAGAAACGTAAATGATATAAATATCAATATATTAAATTAG  
ATTTTGCATAAAAAACAGACTACATAAATACTGTAAACACACATATCCAGTCACTATGG  
CGGCCGCATTAGGCACCCAGGCTTTACACTTTATGCTTCCGGCTCGTATAATGTGTGGA  
TTTTGAGTTAGGATCCGGCGAGATTTTCAGGAGCTAAGGAAGCTAAAAATGGAGAAAAAA  
TCACTGGATATACCACCGTTGATATATCCCAATGGCATCGTAAAGAACATTTTGAGGCAT  
TTCAGTCAGTTGCTCAATGTACCTATAACCAGACCGTTTTCAGCTGGATATTACGGCCTTTT  
TAAAGACCGTAAAGAAAAATAAGCACAAAGTTTTATCCGGCCTTTATTCACATTCCTTGCCC  
GCCTGATGAATGCTCATCCGGAATTCGGTATGGCAATGAAAGACCGTGAGCTGGTGATAT  
GGGATAGTGTTCACCTTGTACACCGTTTTTCCATGAGCAAACTGAAACGTTTTTCATCGC  
TCTGGAGTGAATACCACGACGATTTCCGGCAGTTTCTACACATATATTCGCAAGATGTGG  
CGTGTACGGTGAAAACCTGGCCTATTTCCCTAAAGGGTTTATTGAGAATATGTTTTTCG  
TCTCAGCCAATCCCTGGGTGAGTTTTACCCAGTTTTGATTTAAACGTGGCCAATATGGACA  
ACTTCTTCGCCCCCGTTTTTACCATGGGCAATATTATACGCAAGGCGACAAGGTGCTGA  
TGCCGCTGGCGATTACAGTTTCATCATGCCGTCTGTGATGGCTTCCATGTCCGCAGAATGC  
TTAATGAATTACAACAGTACTGCGATGAGTGGCAGGGCGGGCGTAAACGCGTGGATCCG  
GCTTACTAAAAGCCAGATAACAGTATGCGTATTTGCGCGCTGATTTTTGCGGTATAAGAA  
TATATACTGATATGTATACCCGAAGTATGTCAAAAAGAGGTGTGCTATGAAGCAGCGTAT  
TACAGTGACAGTTGACAGCGACAGCTATCAGTTGCTCAAGGCATATATGATGTCAATATC  
TCCGGTCTGGTAAGCACAACCATGCAGAATGAAGCCCGTCGTCTGCGTGCCGAACGCTGG  
AAAGCGGAAAAATCAGGAAGGGATGGCTGAGGTGCGCCGTTTTATTGAAATGAACGGCTCT  
TTTGCTGACGAGAACAGGGACTGGTGAAATGTCAGTTTAAAGTTTTACACCTATAAAAGAGA  
GAGCCGTTATCGTCTGTTTTGTGGATGTACAGAGTGATATTATTGACACGCCCCGGCGACG  
GATGGTGATCCCCCTGGCCAGTGACCGTCTGCTGTGATGATAAAGTCTCCCGTGAACCTTTA  
CCCCGTGGTGATATCGGGGATGAAAGCTGGCGCATGATGACCACCGATATGGCCAGTGT  
GCCGCTCTCCGTTATCGGGGAAGAAGTGGCTGATCTCAGCCACCGCGAAAAATGACATCAA  
AAACGCCATTAACTGATGTTCTGGGGAATATAAATGTGAGGCTCCGTTATACACAGCCA  
GTCTGCAGGTCGACCATAGTGAAGTGGATATGTTGTGTTTTTACAGTATTATGTAGTCTGTT  
TTTTATGCAAAATCTAATTTAATATATTGATATTTATATCATTTTTACGTTTCTCGTTTCTCAG  
CTTCTTGTACAAAGTGGTGATGGGCGGCGCTCTAGAGGGCCCAAGCTTACGCGTGCAT  
GCGACGTCATAGCTCTCTCCCTATAGTGAGTCGTATTATAAGCTAGGCACTGGCCGTCGT  
TTTACAACGTCGTGACTGGGAAAACCTGCTAGCTTGGGATCTTTGTGAAGGAACCTTACTT  
CTGTGGTGTGACATAATTGGACAACTACCTACAGAGATTTAAAGCTCTAAGGTAATAT  
AAAATTTTTAAGTGATATAATGTGTTAACTAGCTGCATATGCTTGCTGCTTGAGAGTTTT  
GCTTACTGAGTATGATTTATGAAATATTATACACAGGAGCTAGTGATTCTAATTGTTTG  
TGTATTTTAGATTACAGTCCCAAGGCTCATTTTTCAGGCCCCCTCAGTCCTCACAGTCTGTT  
CATGATCATAATCAGCCATACCACATTTGTAGAGGTTTTTACTTGCTTTAAAAAACCTCCC  
ACACCTCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTTGTTGTTAACTTGTTTAT  
TGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTTCAAAATAAAGCATT  
TTTTTCACTGCATTCTAGTTGTGGTTTTGTCCAACTCATCAATGTATCTTATCATGTCTG  
GATCGATCCTGCATTAAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTTGCGTATTGGCT  
GGCGTAATAGCGAAGAGGCCCCGACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATG  
GCCAATGGGACGCGCCCTGTAGCGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCA  
GCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTGCTTTCTTCCCTTCCCT  
TTCTCGCCACGTTCCGCGGCTTTCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGT

Figure 93B

TCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAACTTGATTAGGGTGATGGTTTAC  
GTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCT  
TTAATAGTGGACTCTTGTTCCAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTT  
TTGATTTATAAGGGATTTTGCCGATTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAAC  
AAATATTTAACGCGAATTTTAAACAAAATATTAACGTTTACAATTTTCGCCTGATGCGGTAT  
TTTCTCCTTACGCATCTGTGCGGTATTTACACCCGCATACGCGGATCTGCGCAGCACCAT  
GGCCTGAAATAACCTCTGAAAGAGGAACTTGTTAGGTACCTTCTGAGGCGGAAAGAAC  
AGCTGTGGAATCTGTGTGTCAGTTAGGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAA  
GTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCC  
CAGCAGGCAGAAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCC  
TAACTCCGCCCATCCCGCCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCT  
GACTAATTTTTTTTTTATTTATGTCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGA  
AGTAGTGAGGAGGCTTTTTTGGAGGCCCTAGGCTTTTGCAAAAAGCTTGATTCTTCTGACA  
CAACAGTCTCGAACTTAAGGCTAGAGCCACCATTGATTGAACAAGATGGATTGCACGCAGG  
TTCTCCGGCCGCTTGGGTGGAGAGGCTATTTCGGCTATGACTGGGCACAACAGACAATCGG  
CTGCTCTGATGCCGCCGTGTTCCGGCTGTGACGCGAGGGGCGCCCGGTTCTTTTTGTCAA  
GACCGACCTGTCCGGTGCCCTGAATGAACTGCAGGACGAGGCAGCGCGGCTATCGTGGCT  
GGCCACGACGGGCGTTCTTGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGA  
CTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGATCTCCTGTCTCATCTCACCTTGCTCCTGC  
CGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCTAC  
CTGCCCATTCGACCACCAAGCGAACATCGCATCGAGCGAGCACGTACTCGGATGGAAGC  
CGGTCTTGTCGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACT  
GTTCCGCCAGGCTCAAGGCGCGCATGCCCGACGGCGAGGATCTCGTCGTGACCCATGGCGA  
TGCCTGCTTGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATTCTATCGACTGTGG  
CCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGA  
AGAGCTTGCGCGCGAATGGGCTGACCGCTTCTCGTGCTTTACGGTATCGCCGCTCCCGA  
TTCGACGCGCATCGCCTTCTATCGCCTTCTTGACGAGTCTTCTGAGCGGGACTCTGGGG  
TTCGAAATGACCGACCAAGCGACGCCAACCTGCCATCACGATGGCCGCAATAAAATATC  
TTTATTTTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGAATCGATAGCGATAAGGATCCG  
CGTATGGTGCATCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGCCCCGACA  
CCCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAG  
ACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTGAGAGGTTTTACCGTCTATCACCGAA  
ACGCGCGAGACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTATGATAAT  
AATGGTTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTG  
TTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCTGATAAAT  
GCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTGCGCCCTTAT  
TCCCTTTTTTGCGGCATTTTGCTTCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGT  
AAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAG  
CGGTAAGATCCTTGAGAGTTTTTCGCCCCGAAGAACGTTTTTCCAATGATGAGCACTTTTAA  
AGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTG  
CCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTACCAGTCACAGAAAAGCATCT  
TACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACAC  
TGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCA  
CAACATGGGGGATCATGTAACCTGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCAT  
ACCAAACGACGAGCGTGACACCAGATGCCTGTAGCAATGGCAACAACGTTGCGCAAAC  
ATTAACCTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGC  
GGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGA  
TAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGG  
TAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACG  
AAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTGACACCA  
AGTTTACTCATATATACTTTAGATTGATTTAAACCTTCATTTTTTAATTTAAAAGGATCTA  
GGTGAAGATCCTTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTTCGTTCCA  
CTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCG  
CGTAATCTGCTGCTTGCAAACAAAAAACCCGCTACCAGCGGTGGTTTGTGTCGGGA  
TCAAGAGCTACCAACTCTTTTCCGAAGGTAAGTGGCTTCAGCAGAGCGCGATACCAAA  
TACTTCTCTTAGTGAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCC  
TACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTG  
TCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGTGGGCTGAAC-

Figure 93C

GGGGGGTTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCT  
ACAGCGTGAGCATTGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGCGGACAGGTATCC  
GGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTG  
GTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATG  
CTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCT  
GGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGA  
TAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCG  
CAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGC  
GCGTTGGCCGATTCATTAATGCAGAGCTTGCAATTCGCGCGTTTTTCAATATTATTGAAG  
CATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAA  
ACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCAT  
TATTATCATGACATTAACCTATAAAAAATAGGCGTAGTACGAGGCCCTTCACTCATTAG

217/240

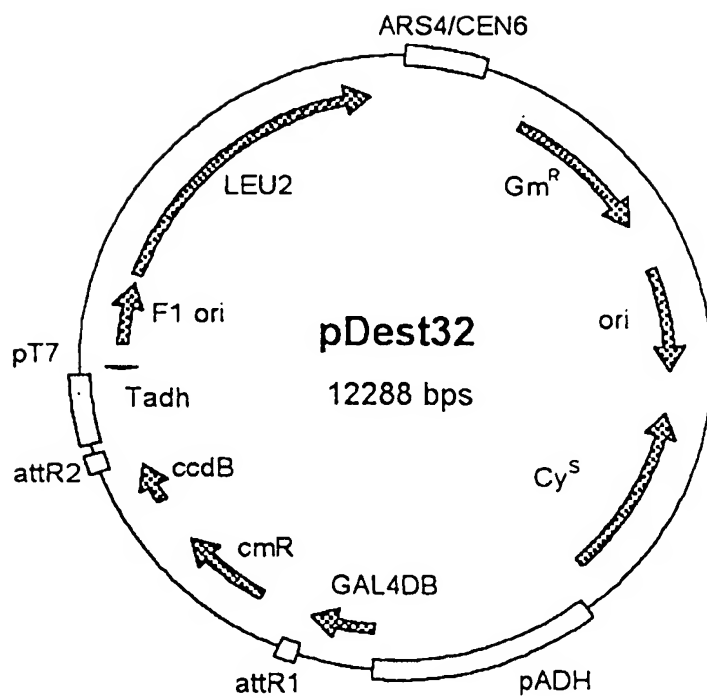


FIGURE 94A

pDEST32 12288 bp

GACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTCATGATAATAATGGTTT  
CTTAGGACGGATCGCTGCTGTAACCTTACACGCGCCTCGTATCTTTAATGATGGAATA  
ATTTGGGAATTTACTCTGTGTTTATTTATTTTATGTTTTGTATTTGGATTTTAGAAAGT  
AAATAAAGAAGGTAGAAGAGTTACGGAATGAAGAAAAAATAAACAAAGGTTTAAAAA  
ATTTCAACAAAAAGCGTACTTTACATATATATTTATTAGACAAGAAAAGCAGATTAAATA  
GATATACATTGATTAACGATAAGTAAATGTAAAATCACAGGATTTTCGTGTGTGGTCT  
TCTACACAGACAAGATGAAACAATTCCGGCATTAATACCTGAGAGCAGGAAGAGCAAGATA  
AAAGGTAGTATTTGTTGGCGATCCCCCTAGAGTCTTTTACATCTTCGGAAAAACAAAACT  
ATTTTTTCTTTAATTTCTTTTTTTTACTTTCTATTTTAAATTTATATATTTATATTA  
ATTTAAATTATAATTATTTTTTATAGCACGTGATGAAAAGGACCCAGGTGGCACTTTTCGG  
GGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCG  
CTCATGAGACAATAACCCCTGATAAATGCTTCAATAATCTGCAGTGCAGGGCCCGTGTC  
TCAAAATCTCTGATGTTACATTGCACAAGATAAAAAATATATCATCATGAACAATAAACT  
GTCTGCTTACATAAACAGTAATACAAGGGGTGTTATGAGCCATATTCAACGGGAAACGTC  
TTGCTGGAGGCCGCGATTAAATTCACATGGATGCTGATTTATATGGGTATAAATGGGC  
TCGGTAGCCAACCACTAGAACTATAGCTAGAGTCTTGGGCGAACAACGATGCTCGCCTT  
CCAGAAAACCGAGGATGCGAACCACTTCATCCGGGGTACAGACCACCGCAAGCGCCGG  
ACGGCCGAGGTCTTCCGATCTCCTGAAGCCAGGGCAGATCCGTGCACAGCACCTTGCCGT  
AGAAGAACAGCAAGGCCGCCAATGCCTGACGATGCGTGGAGACCGAAACCTTGCGCTCGT  
TCGCCAGCCAGGACAGAAATGCCTCGACTTCGCTGCTGCCAAGGTTGCCGGGTGACGCA  
CACCGTGGAAACGGATGAAGGCACGAACCCAGTTGACATAAGCCTGTTCCGTTTCGTAAAC  
TGTAATGCAAGTAGCGTATGCGCTCACGCAACTGGTCCAGAACCTTGACCGAACGCAGCG  
GTGGTAACGGCGCAGTGGCGGTTTTTCATGGCTTGTTATGACTGTTTTTTGTACAGTCTA  
TGCCTCGGGCATCCAAGCAGCAAGCGCGTTACGCCGTGGGTGATGTTGATGTTATGGA  
GCAGCAACGATGTTACGCAGCAGCAACGATGTTACGCAGCAGGGCAGTCGCCCTAAACA  
AAGTTAGGTGGCTCAAGTATGGGCATCATTTCGCACATGTAGGCTCGGCCCTGACCAAGTC  
AAATCCATGCGGGCTGCTCTTGATCTTTTCGGTCTGAGTTCCGAGACGTAGCCACCTAC  
TCCCAACATCAGCCGGACTCCGATTACCTCGGGAACCTTGCTCCGTAGTAAGACATTCATC  
GCGCTTGCTGCCTTCGACCAAGAAGCGGTTGTTGGCGCTCTCGCGGCTTACGTTCTGCCC  
AGGTTTGAGCAGCCGCGTAGTGAGATCTATATCTATGATCTCGCAGTCTCCGGCGAGCAC  
CGGAGGCAGGGCATTGCCACCGCGCTCATCAATCTCCTCAAGCATGAGGCCAACGCGCTT  
GGTGCTTATGTGATCTACGTGCAAGCAGATTACGGTGACGATCCCGCAGTGGCTCTCTAT  
ACAAAGTTGGGCATACGGGAAGAAGTGATTCGCACTTTGATATCGACCCAAGTACCGCCACC  
TAACAATTTCGTTCAAGCCGAGATCGGCTTCCCGGCCAATAGGTTGTATTGATGTTGGAC  
GAGTCGGAATCGCAGACCGGATACAGGATCTTGCCATCCTATGGAACCTGCCTCGGTGAGT  
TTTCTCCTTCATTACAGAAACGGCTTTTTCAAAAATATGGTATTGATAATCCTGATATGA  
ATAAATTGCAGTTTCATTTGATGCTCGATGAGTTTTTCTAATCAGAATTGGTTAATTGGT  
TGTAACACTGGCAGAGCATTACGCTGACTTGACGGGACGGCGNCATGACCAAAATCCCTT  
AACGTGAGTTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTT  
GAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAACACCGCTACCAG  
CGGTGGTTTGTGTTGCCGATCAAGAGCTACCAACTTTTTTCCGAAGGTAAGTGGCTTCA  
GCAGAGCGCAGATACCAAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCA  
AGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTG  
CCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGG  
CGCAGCGGTCCGGCTGAACGGGGGGTTCGTGCACACAGCCAGCTTGGAGCGAACGACCT  
ACACCGAACTGAGATACCTACAGCGTGAGCATTGAGAAAGCGCCACGCTTCCCGAAGGGA  
GAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGC  
TTCCAGGGGGGAACGCCTGGTATCTTTATAGTCTGTGCGGTTTCGCCACCTCTGACTTG  
AGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGAGCCTATGGAAAAACGCCAGCAACG  
CGGCCTTTTTTACGGTTCTTGCCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCTGCGT  
TATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCC  
GCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATAC  
GCAAAACCGCCTCTCCCGCGCGTTGGCCGATTCAATTAATGCAGCTGGCACGACAGGTTTC  
CCGACTGGAAAGCGGCGAGTGAGCGCAACGCAATTAATGTGAGTTACCTCACTCATTAGG  
CACCCCAGGCTTTACACTTTATGCTTCCGGCTCCTATGTTGTGTGGAATTGTGAGCGGAT  
AACAATTTACACAGGAAACAGCTATGACCATGATTACGCCAAGCTCGGAATTAACCTC-

FIGURE 94B



ACTAAAGGGAACAAAAGCTGGTACCGATCCCGAGCTTTGCAAATTAAAGCCTTCGAGCGT  
CCCCAAAACCTTCTCAAGCAAGGTTTTCAGTATAATGTTACATGCGTACACGCGTCTGTAC  
AGAAAAAAGAAAAATTTGAAATATAAATAACGTTCTTAATACTAACATAACTATAAAA  
AAATAAATAGGGACCTAGACTTCAGGTTGTCTAACTCCTTCTTTCGTTAGAGCGGAT  
GTGGGGGGAGGGCGTGAATGTAAGCGTGACATAACTAATTACATGATATCGACAAAGGAA  
AAGGGGCCTGTTTACTCACAGGCTTTTTTCAAGTAGGTAATTAAGTCGTTTCTGTCTTTT  
TCCTTCTTCAACCCACCAAAGGCCATCTTGGTACTTTTTTTTTTTTTTTTTTTTTTTTTT  
TT  
TTTTTTTTTTCATAGAAATAATACAGAAGTAGATGTTGAATTAGATTAACTGAAGATATAT  
AATTTATTGGAATAACATAGAGCTTTTTGTTGATGCGCTTAAGCGATCAATTCACAAC  
ACCACCAGCAGCTCTGATTTTTTCTTCAGCCAACCTGGAGACGAATCTAGCTTTGACGAT  
AACTGGAACATTTGGAATCTACCCTTACCCAAGATCTTACCGTAACCGGCTGCCAAAGT  
GTCAATAACTGGAGCAGTTTCTTAGAAGCAGATTTCAAGTATTGGTCTCTCTGTCTTC  
TGGGATCAATGTCCACAATTTGTCCAAGTTCAAGACTGGCTTCCAGAAATGAGCTTGTG  
CTTGTGGAAGTATCTCATACCAACCTTACCGAAATAACCTGGATGGTATTTATCCATGTT  
AATTCTGTGGTGATGTTGACCACCGGCATACCTCTACCACCGGGGTGCTTTCTGTGCTT  
ACCGATACGACCTTACCAGGCTGAGACGTGACCTCTGTGCTTTCTAGTCTTAGTGAATCT  
GGAAGGCATTCTGATTAGTTGGATGATTGTTCTGGGATTTAATGCAAAAATCACTTAAG  
AAGGAAAATCAACGGAGAAAGCAAACGCCATCTTAAATATACGGGATACAGATGAAAGGG  
TTTGAACCTATCTGGAATAAGCATTAAACAAGCGAAAAACTGCGAGGAAAATGTTTGC  
GTCTCTGCGGGCTATTACGCGCCAGAGGAAAATAGGAAAAATAACAGGGCATTAGAAAA  
ATAATTTTGATTTTGGTAATGTGTGGGTCTGGTGACAGATGTTACATTGGTTACAGTA  
CTCTTGTTTTGCTGTGTTTTTCGATGAATCTCCAAATGGTTGTTAGCACATGGAAGAG  
TCACCGATGCTAAGTTATCTCTATGTAAGCTACGTGGCGTGACTTTTGATGAAGCCGCAC  
AAGAGATACAGGATTGGCAACTGCAATAGAAATCTGGGGATCCCCCTCGAGATCCGGGA  
TCGAAGAAAATGATGGTAAATGAAATAGGAAATCAAGGAGCATGAAGGCAAAAGACAAATA  
TAAGGGTCAACGAAAAATAAAGTGAAGAGTGTGATATGATGATTTGGCTTTGCGGCG  
TTGCCGGCCCCGGCGATAACGCTGGGCGTGAGGCTGTGCCGGCGGAGTTTTTTGCGCCTG  
CATTTTCCAAGTTTTACCCTGCGCTAAGGGGCGAGATTGGAGAAGCAATAAGAATGCCGG  
TTGGGGTTGCGATGATGACGACCACGACAACCTGGTGTCTATTATTTAAGTTGCCGAAAGAA  
CCTGAGTGCATTTGCAACATGAGTATACTAGAAGAATGAGCCAAGACTTGCGAGACGCGA  
GTTTGCCGGTGGTGCGAACAATAGAGCGACCATGACCTTGAAGGTGAGACGCGCATAACC  
GCTAGAGTACTTTGAAGAGGAAACAGCAATAGGGTTGCTACCAGTATAAATAGACAGGTA  
CATACAACACTGGAAATGGTTGTCTGTTGAGTACGCTTTCAATTCATTTGGGTGTGCAC  
TTTATTATGTTACAATATGGAAGGGAACCTTTACACTTCTCCTATGCACATATATTAATTA  
AAGTCCAATGCTAGTAGAGAAGGGGGTAACACCCCTCCGCGCTCTTTTCCGATTTTTTT  
CTAAACCGTGAATATTTTCGGATATCCTTTTGTGTTTCCGGGTGTACAATATGGACTTC  
CTCTTTTCTGGCAACCAACCCATACATCGGGATTCTTATAATACCTTCGTTGGTCTCCC  
TAACATGTAGGTGGCGGAGGGGAGATATAACAATAGAACAGATACCAGACAAGACATAATG  
GGCTAAACAAGACTACACCAATTACACTGCCTCATTGATGGTGGTACATAACGAACATAAT  
ACTGTAGCCCTAGACTTGATAGCCATCATCATATCGAAGTTTCACTACCCTTTTTCCATT  
TGCCATCTATTGAAGTAATAATAGGCGCATGCAACTTCTTTCTTTTTTTTTTCTTTTCTC  
TCTCCCCCGTTGTTGTCTCACCATATCCGCAATGACAAAAAAATGATGGAAGACACTAA  
AGGAAAAAATTAACGACAAAGACAGCACCACAGATGTCGTTGTTCCAGAGCTGATGAGG  
GGTATCTTGAACACACGAACTTTTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCT  
AGCAACGGTATACGGCCTTCTTCCAGTTACTTGAATTTGAAATAAAAAAAGTTTGGCCG  
TTTGCTATCAAGTATAAATAGACCTGCAATTATTAATCTTTTGTTCCTCGTCATTGTTT  
TCGTTCCCTTTCTTCTTGTCTTTTTCTGCACAATATTTCAAGCTATACCAAGCATAC  
AATCAACTCCAAGCTTGAAGCAAGCCTCCTGAAAGATGAAGCTACTGTCTTCTATCGAAC  
AAGCATGCGATATTTGCCGACTTAAAAAGCTCAAGTGCTCCAAAGAAAAACCGAAGTGCG  
CCAAGTGCTGAAGAACAACCTGGGAGTGTGCTACTCTCCCAAAACCAAAAGGTCTCCG  
TGACTAGGGCACATCTGACAGAAGTGAATCAAGGCTAGAAAGACTGGAACAGCTATTTC  
TACTGATTTTCTCTGAGAAGACCTTGACATGATTTTGAAAATGGATTCTTTACAGGATA  
TAAAGACTTGTGTTAACAGGATTATTTGTACAAGATAATGTGAATAAAGATGCCGTCACAG  
ATAGATTGGCTTCAGTGGAGACTGATATGCCTTAACATTGAGACAGCATAGAATAAGTG  
CGACATCATCATCGGAAGAGAGTAGTAACAAAGGTCAAAGACAGTTGACTGTATCGTCGA  
GGTCAATCAAACAAGTTTGTACAAAAAAGCTGAACGAGAAACGTAAATGATATAAATA-

TCAATATATTAAATTAGATTTTGCATAAAAAACAGACTACATAATACTGTAAAAACACAAC  
ATATCCAGTCACTATGGCGGCCGCTAAGTTGGCAGCATCACCCGACGCACTTTGCGCCGA  
ATAAATACCTGTGACGGAAGATCACTTCGCAGAAATAAATAAATCCTGGTGTCCCTGTTGA  
TACCGGGAAGCCCTGGGCCAACTTTTGGCGAAAAATGAGACGTTGATCGGCACGTAAGAGG  
TTCCAACCTTTCACCATAATGAAATAAGATCACTACCGGGCGTATTTTTTGTAGTTATCGAG  
ATTTTCAGGAGCTAAGGAAGCTAAAAATGGAGAAAAAATCACTGGATATACCACCGTTGA  
TATATCCCAATGGCATCGTAAAGAACATTTTGAGGCATTTTCAGTCAGTTGCTCAATGTAC  
CTATAACCAGACCGTTTCAGCTGGATATTACGGCCTTTTTTAAAGACCGTAAAGAAAAATAA  
GCACAAGTTTTATCCGGCCTTTATTCACATTCCTGCCCCGCTGATGAATGCTCATCCGGA  
ATTCGGTATGGCAATGAAAGACGGTGAGCTGGTGATATGGGATAGTGTTCAACCCTTGTTA  
CACCGTTTTCCATGAGCAAACCTGAAACGTTTTTCATCGCTCTGGAGTGAATACCACGACGA  
TTTCCGGCAGTTTTCTACACATATATTCGCAAGATGTGGCGTGTTACGGTGAAAAACCTGGC  
CTATTTCCCTAAAGGGTTTTATTGAGAATATGTTTTTCGTCTCAGCCAATCCCTGGGTGAG  
TTTTCAACGTTTTTGATTTAAACGTGGCCAAATATGGACAACCTTCTTCGCCCCCGTTTTTAC  
CATGGGCAAATATTATACGCAAGGCGACAAGGTGCTGATGCCGCTGGCGATTTCAGGTTCA  
TCATGCCGCTCTGTGATGGCTTCCATGTCGGCAGAAATGCTTAATGAATTACAACAGTACTG  
CGATGAGTGGCAGGGCGGGGCGTAATCTAGAGGATCCGGCTTACTAAAAAGCCAGATAACA  
GTATGCGTATTTGCGCGCTGATTTTTGCGGTATAAGAATATATACTGATATGTATACCCG  
AAGTATGTCAAAAAGAGGTGTGCTATGAAGCAGCGTATTACAGTGACAGTTGACAGCGAC  
AGCTATCAGTTGCTCAAGGCATATATGATGTCAATATCTCCGGTCTGGTAAGCACAAACA  
TGCGAATGAAGCCCGTCTGCTGCGTGCCGAACGCTGGAAAGCGGAAAAATCAGGAAGGGA  
TGGCTGAGGTGCGCCCGTTTTATTGAAATGAACGGCTCTTTTGCTGACGAGAACAGGGACT  
GGTGAATGCAAGTTTAAAGTTTTACACCTATAAAAGAGAGAGCCGTTATCGTCTGTTTGTG  
GATGTACAGAGTGATATTATTGACACGCCCCGGGCGACGGATGGTGATCCCCCTGGCCAGT  
GCACGTCTGCTGTGATGATAAAGTCTCCCGTGAACCTTTACCCGGTGGTGATATCGGGGAT  
GAAAGCTGGCGCATGATGACCACCGATATGGCCAGTGTCGGGTCTCCGTTATCGGGGAA  
TAGATGGCTGATCTCAGCCACCGCGAAAAATGACATCAAAAACGCCATTAACTGATGTTT  
TGGGGAATATAAATGTGAGGCTCCCTTATACACAGCCAGTCTGCAGGTCGACCATAGTGA  
CTGGATATGTTGTGTTTTACAGTATTATGTAGTCTGTTTTTTATGCAAAATCTAATTTAA  
TATATTGATATTTATATCATTTTTACGTTTTCTCGTTTCAGCTTTCTTGATACAAAGTGGTTG  
ATGGCCGCTAAGTAAGTAAGACGTCGAGCTCTAAGTAAGTAACGGCCGCCACCGCGGTGG  
AGCTTTGGACTTCTTCGCCAGAGGTTTGGTCAAGTCTCCAATCAAGGTTGTGCGCTTGTG  
TACCTTGCCAGAAATTTACGAAAAGATGGAAGGGTCAAATCGTTGGTAGATACGTTGT  
TGACACTTCTAAATAAGCGAATTTCTTATGATTTATGATTTTTATTATTAAATAAGTTAT  
AAAAAAATAAGTGATACAAATTTTAAAGTGACTCTTAGGTTTTAAACGAAAATTTCTT  
GTTCTTGAGTAACCTCTTCTGTAGGTGAGGTTGCTTTCTCAGGTATAGCATGAGGTGCG  
TCTTATTGACCACACCTCTACCGGCATGCCGAGCAAATGCCGCAATCGCTCCCCATTT  
CACCCAATTGTAGATATGCTAACTCCAGCAATGAGTTGATGAATCTCGGTGTGATTTTA  
TGTCCTCAGAGGACAATACCTGTTGTAATCGTTCTTCCACACGGATCCCAATTCGCCCTA  
TAGTGAGTCGTATTACAATTCAGTGGCGTCGTTTTACAACGTCGTGACTGGGAAAAACC  
TGGCGTTACCCAACTTAATCGCCTTGACGACATCCCCCTTTCGCCAGCTGGCGTAATAG  
CGAAGAGGCCCCGACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGAC  
GCGCCCTGTAGCGGCGCATTAAGCGCGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCT  
ACACTTGCCAGCGCCCTAGCGCCGCTCCTTTTCGCTTTCTTCCCTTCTTTCTCGCCACG  
TTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCGGATTTAGT  
GCTTTACGGCACCTCGACCCCAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCA  
TCGCCCTGATAGACGTTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGA  
CTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTTTTGATTTATAA  
GGGATTTTGGCGATTTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAAC  
GCGAATTTTAACAAAATATTAAACGTTTACAATTTCTGATGCGGTATTTTCTCCTTACGC  
ATCTGTGCGGTATTTACACCGCATATCGACCGGTGAGGAGAACTTCTAGTATATCCAC  
ATACCTAATATTATGCTTATTAAAAATGGAATCGGAACAATTACATCAAAATCCACAT  
TCTCTTCAAAATCAATTGTCTGTACTTCTTGTTCATGTGTGTTCAAAAACGTTATATT  
TATAGGATAATTATACTCTATTTCTCAACAAGTAATTGGTTGTTTGGCCGAGCGGTCTAA  
GGCGCCTGATTCAAGAAATATCTTGACCGCAGTTAACTGTGGGAATACTCAGGTATCGTA  
AGATGCAAGAGTTGCAATCTCTAGCAACCATTATTTTTTTCTCAACATAACGAGAACA  
CACAGGGGCGCTATCGCACAGAATCAAATTCGATGACTGGAAATTTTTTGTAAATTCAG  
AGGTGCGCTGACGCATATACCTTTTTTCAACTGAAAAATGGGAGAAAAAGGAAAGGTGAG-

FIGURE 94D



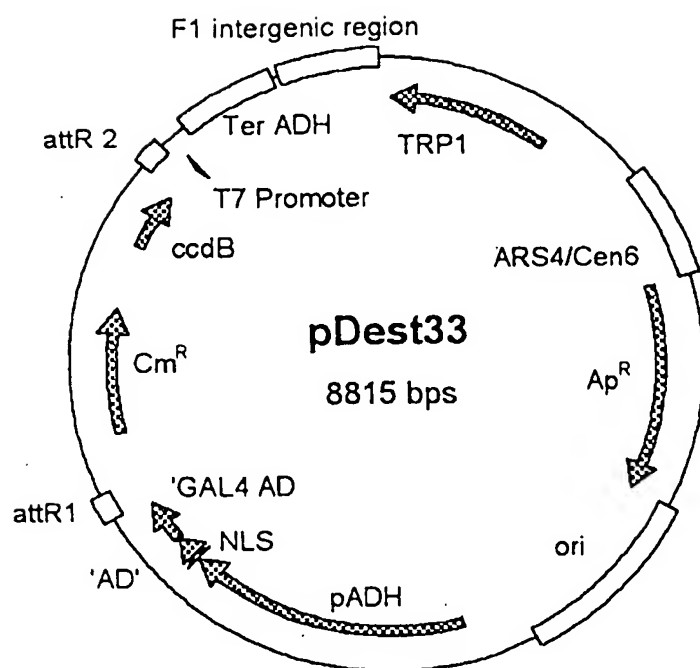


FIGURE 95A

pDEST33 8815 bp

GCCTTACGCATCTGTGCGGTATTTACACCCGAGGCAAGTGCACAAACAATACTTAAATA  
AATACTACTCAGTAATAACCTATTTCTTAGCATTTTTGACGAAATTTGCTATTTTGTAG  
AGTCTTTTACACCATTTGTCTCCACACCTCCGCTTACATCAACACCAATAACGCCATTTA  
ATCTAAGCGCATCACCAACATTTTCTGGCGTCAGTCCACCAGCTAACATAAAATGTAAGC  
TTTCGGGGCTCTCTTGCTTCCAACCCAGTCAGAAATCGAGTTCCAATCCAAAAGTTTAC  
CTGTCCACCTGCTTCTGAATCAAACAAGGGAATAAACGAATGAGGTTTCTGTGAAGCTG  
CACTGAGTAGTATGTTGCAGTCTTTTGGAAATACGAGTCTTTAATAACTGGCAAACCGA  
GGAATCTTTGGTATTCTTGCCACGACTCATCTCCATGCAGTTGGACGATATCAATGCCGT  
AATCATTGACCAGAGCCAAAACATCCTCCTTAGGTTGATTACGAAACACGCCAACCAAGT  
ATTTCCGAGTGCCTGAACTATTTTTATATGCTTTTACAAGACTTGAAATTTTCTTGCAA  
TAACCGGGTCAATTGTTCTCTTTCTATTGCGCACACATATAATACCCAGCAAGTCAGCAT  
CGGAATCTAGAGCACATTCTGCGGCTCTGTGCTCTGCAAGCCGCAAACCTTTCACCAATG  
GACCAGAACTACCTGTGAAATTAATAACAGACATACTCCAAGCTGCCTTTGTGTGCTTAA  
TCACGTATACTCACGTGCTCAATAGTCACCAATGCCCTCCCTCTTGCCCTCTCCTTTTC  
TTTTTTTCGACCGAATTAATTCTTAATCGGCAAAAAAGAAAGCTCCGGATCAAGATTGT  
ACGTAAGGTGACAAGCTATTTTTCAATAAAGAATATCTTCCACTACTGCCATCTGGCGTC  
ATAACTGCAAAGTACACATATATTACGATGCTGTCTATTAAATGCTTCTATATTATATA  
TATAGTAATGTCGTTTATGGTGCACCTCTCAGTACAATCTGCTCTGATGCCGATAGTTAA  
GCCAGCCCCGACACCCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGG  
CATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTGAGAGTTTTCAC  
CGTCATCACCGAAACGCGCGAGACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTA  
ATGTCATGATAATAATGGTTTCTTAGGACGGATCGCTTGCCTGTAACCTACACGCGCCTC  
GTATCTTTTAAATGATGGAATAATTTGGGAATTTACTCTGTGTTTATTTATTTTATGTTT  
TGTATTTGGATTTTAGAAAGTAAATAAAGAAGGTAGAAGAGTTACGGAATGAAGAAAAA  
AAATAAACAAAGGTTTAAAAAATTTCAACAAAAAGCGTACTTTACATATATATTTATTAG  
ACAAGAAAAAGCAGATTAAATAGATATACATTGATTAACGATAAGTAAATGTAAATCA  
CAGGATTTTCTGTGTGTTCTTCTACACAGACAAGATGAAACAATTCGGCATTAAATACCT  
GAGAGCAGGAAGAGCAAGATAAAAGGTAGTATTTGTTGGCGATCCCCCTAGAGTCTTTTA  
CATCTTCGGAAAAACAAAACTATTTTTCTTTAATTTCTTTTTTACTTTCTATTTTTAA  
TTTATATATTTATATTAAAAAATTTAAATTATAATTATTTTTATAGCACGTGATGAAAAG  
GACCCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTCTTAA  
ATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATAT  
TGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTGCGCCCTTATTCCTTTTTTTCG  
GCATTTTGCCTTCTGTTTTTGTCTACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAA  
GATCAGTTGGGTGCACGAGTGGGTACATCGAAGTGGATCTCAACAGCGGTAAAGATCCTT  
GAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGT  
GGCGCGGTATTATCCCGTATTGACGCGGGCAAGAGCAACTCGGTGCGCGCATACACTAT  
TCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATG  
ACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTA  
CTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTTTCAACATGGGGGAT  
CATGTAACCTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAG  
CGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAACCTGGCGAA  
CTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCA  
GGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCC  
GGTGAGCGTGGGTCTCGCGGTATCATTTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGT  
ATCGTAGTTATCTACACGACGGGCAGTCAGGCAACTATGGATGAACGAAATAGACAGATC  
GCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTGACACCAAGTTTACTCATAT  
ATACTTTAGATTGATTTAAACTTCATTTTTTAATTTAAAGGATCTAGGTGAAGATCCTT  
TTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTTCGTTCCACTGAGCGTCAGAC  
CCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGC  
TTGCAAAACAAAAACCACCGCTACCAGCGGTGGTTTGTGTTGCCGGATCAAGAGCTACCA  
ACTTTTTTCCGAAGGTAACCTGGCTTCAGCAGAGCGCAGATACCAATACTGTCTCTTA  
GTGTAGCCGTAGTTAGGCCACCCTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCT  
CTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTG  
GACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTGGGGCTGAACGGGGGGTTCTGTG  
ACACAGCCCAGCTTGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCAT-

FIGURE 95B

TGAGAAAAGCGCCACGCTTCCCAGGAGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGG  
GTCCGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGGAACGCCTGGTATCTTTATAGT  
CCTGTCCGGGTTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGG  
CCGAGCCTATGGAAAAACGCCAGCAACGCGGCCCTTTTACGGTTCCTGGCCTTTTGCTGG  
CCTTTTGCTCACATGTTCTTCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACC  
GCCTTTGAGTGAGCTGATACCGCTCGCCGAGCCGAACGACCGAGCGCAGCGAGTCAGTG  
AGCGAGGAAGCGGAAGAGCGCCCAATACGCAAAACCGCCTCTCCCGCGCGTTGGCCGATT  
CATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCA  
ATTAATGTGAGTTACCTCACTCATTAGGCACCCAGGCTTTACACTTTATGCTTCCGGCT  
CCTATGTTGTGTGGAATTGTGAGCGGATAACAATTTACACAGGAAACAGCTATGACCAT  
GATTACGCCAAGCTCGGAATTAACCCTCACTAAAGGGAACAAAAGCTGGGTACCGGGCCC  
CCCCTCGAGATCCGGGATCGAAGAAATGATGGTAAATGAAATAGGAAATCAAGGAGCATG  
AAGGCAAAAGACAAATATAAGGGTCGAACGAAAAATAAGTGAAAAGTGTTGATATGATG  
TATTTGGCTTTGCGGCGCCGAAAAACGAGTTTACGCAATTGCACAATCATGTGACTCT  
GTGGCGGACCCGCGCTCTTGCCGGCCCGCGGATAACGCTGGGCGTGAGGCTGTGCCCGGC  
GGAGTTTTTTGCGCCTGCATTTTCCAAGGTTTACCCTGCGCTAAGGGGCGAGATTGGAGA  
AGCAATAAGAATGCCGGTTGGGGTTGCGATGATGACGACCACGACAACTGGTGTCATTAT  
TTAAGTTGCCGAAAGAACCTGAGTGCATTTGCAACATGAGTATACTAGAAGAATGAGCCA  
AGACTTGCGAGACGCGAGTTTGCCGGTGGTGCGAACAATAGAGCGACCATGACCTTGAAG  
GTGAGACGCGCATAACCGCTAGAGTACTTTGAAGAGGAAACAGCAATAGGGTTGCTACCA  
GTATAAATAGACAGGTACATAACAACCTGGAATGTTGTCTGTTTGTGAGTACGCTTCAA  
TTCATTTGGGTGTGCACTTTTATTATGTTACCAATATGGAAGGGAACCTTTACACTTCTCCTA  
TGCACATATATTAATTAAAGTCCAATGCTAGTAGAGAAGGGGGGTAAACCCCCTCCGCGC  
TCTTTTCCGATTTTTTTCTAAACCGTGGAATATTTTCGGATATCCTTTTGTGTTTCCGGG  
TGTACAATATGGACTTCCTCTTTCTGGCAACCAACCCATACATCGGGATTCTCTATAAT  
ACCTTCGTTGGTCTCCCTAACATGTAGGTGGCGGAGGGGAGATATACAATAGAACAGATA  
CCAGACAAGACATAATGGGCTAAACAAGACTACACCAATTACACTGCCTCATTGATGGTG  
GTACATAACGAACATAACTGTAGCCCTAGACTTGATAGCCATCATCATATCGAAGTTTC  
ACTACCCTTTTTCCATTTGCCATCTATTGAAGTAATAATAGGCGCATGCAACTTCTTTTC  
TTTTTTTTTTCTTTCTCTCTCCCCGTTGTTGTCTCACCATATCCGCAATGACAAAAAA  
ATGATGGAAGACACTAAAGGAAAAAATTAACGACAAAGACAGCACCAACAGATGTCGTTG  
TTCCAGAGCTGATGAGGGGTATCTTCGAACACAGAACTTTTTCTTCTTCTTATTACG  
CACACTACTCTCTAATGAGCAACGGTATACGGCCTTCTTCCAGTTACTTGAATTTGAAA  
TAAAAAAGTTTGCCGCTTTGCTATCAAGTATAAATAGACCTGCAATTATTAATCTTTTG  
TTTCTCGTCATTGTTCTCGTTCCCTTTCTTCTTCTTTCTTTTCTGCAATATTTTCA  
AGCTATACCAAGCATACAATCAACTCCAAGCTTATGCCAAGAAGAAGCGGAAGGTCTCG  
AGCGGCGCCAATTTTAATCAAAGTGGGAATATTGCTGATAGCTCATTGTCCTTCACTTTC  
ACTAACAGTAGCAACGGTCCGAACCTCATAACAACCTCAAACAAATTCTCAAGCGCTTTCA  
CAACCAATTGCCTCCTCTAACGTTTATGATAACTTCATGAATAATGAAATCACGGCTAGT  
AAAATTGATGATGGTAATAATTCAAACCACTGTCACTGGTTGGACGGACCAAACTGCG  
TATAACGCGTTTGAATCACTACAGGGATGTTTAAATACCACTACAATGGATGATGTATAT  
AACTATCTATTGATGATGAAGATACCCACCAAAACCAAAAAAGAGGGTGGGTGCAAT  
CAAACAAGTTTGTACAAAAAGCTGAACGAGAAACGTAAATGATATAAATATCAATATA  
TTAAATTAGATTTTGCATAAAAAACAGACTACATAATACTGTAAACACAACATATCCAG  
TCACTATGGCGGCCGCTAAGTTGGCAGCATCACCCGACGCACTTTGCGCCGAATAAATAC  
CTGTGACGGAAGATCACTTCGCAGAATAAATAAATCCTGGTGTCCCTGTTGATACCGGGA  
AGCCCTGGGCCAACTTTTGGCGAAAATGAGACGTTGATCGGCACGTAAGAGGTTCCAAC  
TTCAACATAATGAAATAAGATCACTACCGGGCGTATTTTTTGTGATTATCGAGATTTTCAG  
GAGCTAAGGAAGCTAAATGGAGAAAAAATCACTGGATATACCACCGTTGATATATCCC  
AATGGCATCGTAAAGAACATTTTGAAGCATTTTCACTCAGTTGCTCAATGTACCTATAACC  
AGACCGTTTCACTGGATATTACGGCCTTTTTTAAAGACCGTAAAGAAAAATAAGCACAACT  
TTTATCCGGCCTTTATTACATTTCTGCCCCGCTGATGAATGCTCATCCGGAATTCGGTA  
TGGCAATGAAAGACGGTGAGCTGGTGATATGGGATAGTGTTACCCCTGTTACACCGTTT  
TCCATGAGCAAACTGAAACGTTTTCATCGCTCTGGAGTGAATACACGACGATTTCCGGC  
AGTTTCTACACATATATTGCAAGATGTGGCGTGTTACGGTGAAAAACCTGGCCTATTTCC  
CTAAAGGGTTTATTGAGAATATGTTTTCTGCTCTCAGCCAATCCCTGGGTGAGTTTACCA  
GTTTTGATTTAAACGTGGCCAATATGGACAACCTTCTCGCCCCGTTTTTACCATGGGCA  
AATATTATACGCAAGGCGACAAGGTGCTGATGCCGCTGGCGATTTCAGGTTTCATGCGC-

FIGURE 95C

TCTGTGATGGCTTCCATGTCGGCAGAATGCTTAATGAATTACAACAGTACTGCGATGAGT  
GGCAGGGCGGGGCGTAATCTAGAGGATCCGGCTTACTAAAAGCCAGATAACAGTATGCGT  
ATTTGCGCGCTGATTTTTGCGGTATAAGAATATATACTGATATGTATACCCGAAGTATGT  
CAAAAAGAGGTGTGCTATGAAGCAGCGTATTACAGTGACAGTTGACAGCGACAGCTATCA  
GTTGCTCAAGGCATATATGATGTCAATATCTCCGGTCTGGTAAGCACAAACCATGCAGAAT  
GAAGCCCGTCGTCTGCGTGCCGAACGCTGGAAAGCGGAAAATCAGGAAGGGATGGCTGAG  
GTCGCCCCGTTTTATTGAAATGAACGGCTCTTTTGCTGACGAGAACAGGGACTGGTGAAAT  
GCAGTTTAAAGTTTTACACCTATAAAAGAGAGAGCCGTTATCGTCTGTTTGTGGATGTACA  
GAGTGATATTATTGACACGCCCCGGGCGACGGATGGTGATCCCCCTGGCCAGTGCACGTCT  
GCTGTCAGATAAAGTCTCCCGTGAACTTTACCCGGTGGTGATATCGGGGATGAAAGCTG  
GCGCATGATGACCACCGATATGGCCAGTGTGCCGGTCTCCGTTATCGGGGAAGAAGTGGC  
TGATCTCAGCCACCGCGAAAATGACATCAAAAACGCCATTAACTGATGTTCTGGGGAAT  
ATAAATGTCAGGCTCCGTTATACACAGCCAGTCTGCAGGTGCGACCATAGTGAAGTGGATAT  
GTTGTGTTTTTACAGTATTATGTAGTCTGTTTTTTATGCAAAATCTAATTTAATATATTGA  
TATTTATATCATTTTACGTTTCTCGTTTCAGCTTTCTTGTACAAAGTGGTTTGTGGCCGC  
TAAGTAAGTAAGACGTCGAGCTCCCTATAGTGAGTCGTATTACACTGGCCGTCGTTTTAC  
AACGTCGTGACTGGGAAAACACCGGTGAGCTCTAAGTAAGTAACGGCCGCCACCGCGGTG  
GAGCTTTGGACTTCTTCGCCAGAGGTTTGGTCAAGTCTCCAATCAAGGTTGTCCGGCTTGT  
CTACCTTGCCAGAAAATTTACGAAAAGATGAAAAGGGTCAAATCGTTGGTAGATACGTTG  
TTGACACTTCTAAATAAGCGAATTTCTTATGATTTATGATTTTTATTATTAAATAAGTTA  
TAAAAAAAATAAGTGTATACAAATTTTAAAGTGAAGTCTTAGGTTTTAAACGAAAATTTCT  
TGTTCTTGAGTAACTCTTTCCTGTAGGTGAGTTGCTTTCTCAGGTATAGCATGAGGTGCG  
CTCTTATTGACCACACCTCTACCGGCATGCCGAGCAAATGCCTGCAAATCGCTCCCCATT  
TCACCCAATTGTAGATATGCTAACTCCAGCAATGAGTTGATGAATCTCGGTGTGATTTTT  
ATGTCCTCAGAGGACAATACCTGTTGTAATCGTTCTTCCACACGGATCCGCATCAGGCGA  
AATTGTAAACGTTAATATTTTGTAAATTCGCGTTAAATATTTGTTAAATCAGCTCATT  
TTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAATCAAAGAATAGACCGAGAT  
AGGGTTGAGTGTGTTCCAGTTTGGACAAGAGTCCACTATTAAAGAACGTGGACTCCAA  
CGTCAAAGGGCGAAAACCGTCTATCAGGGCGATGGCCCACTACGTGAACCATCACCCTA  
ATCAAGTTTTTTGGGGTTCGAGGTGCCGTAAAGCACTAAATCGGAACCCTAAAGGGAGCCC  
CCGATTTAGAGCTTGACGGGAAAGCCGGCGAACGTGGCGAGAAAGGAAGGGAAGAAAGC  
GAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGGTCACGCTGCGCGTAACCACCAC  
ACCCGCCGCGCTTAATGCGCCGCTACAGGGCGCGTCCCATTCGCCATTCACTGCA

FIGURE 95D

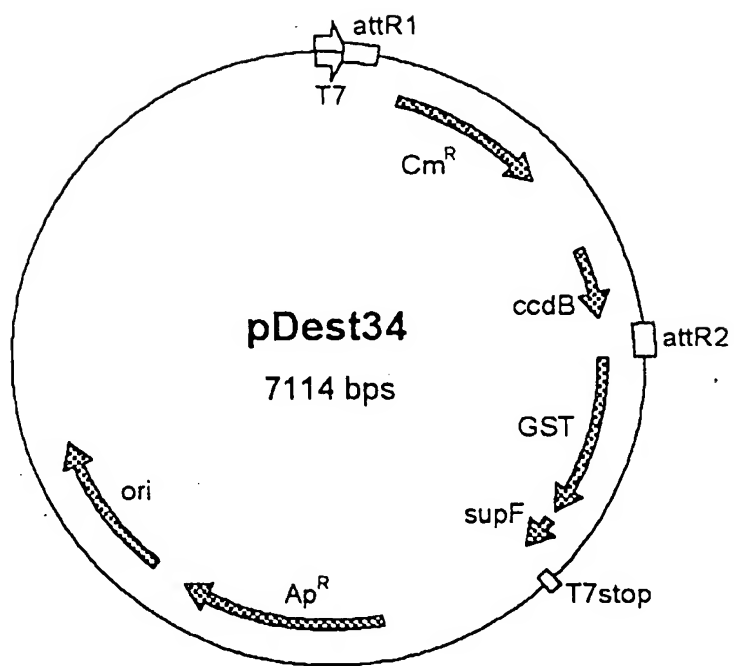


FIGURE 96A



## pDEST34 7114 bp

<u>Location (Base Nos.)</u>	<u>Gene Encoded</u>
195..71	attR1
304..963	CmR
1305..1610	ccdB
1651..1775	attR2
1780..2472	GST
2675..2720	T7stop
3334..4194	ampR
4343..4982	ori

ATCGAGATCTCGATCCCGCGAAATTAATACGACTCACTATAGGGAGACCACAACGGTTTC  
 CCTCTAGATCACAGTTTGTACAAAAAGCTGAACGAGAAACGTAAAATGATATAAATAT  
 CAATATATTAAATTAGATTTTGCATAAAAAACAGACTACATAATACTGTAAAACACAACA  
 TATCCAGTCACTATGGCGGCCCGCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGC  
 TCGTATAATGTGTGGATTTTGTAGTTAGGATCCGGCGAGATTTTCAGGAGCTAAGGAAGCT  
 AAAATGGAGAAAAAATCACTGGATATACCACCGTTGATATATCCCAATGGCATCGTAAA  
 GAACATTTTGTAGGCATTTTCAGTCAGTTGCTCAATGTACCTATAACCAGACCGTTCAGCTG  
 GATATTACGGCCTTTTTAAAGACCGTAAAGAAAAATAAGCACAAGTTTTATCCGGCCTTT  
 ATTCACATTTCTGCCCCGCTGATGAATGCTCATCCGGAATTCGATGGCAATGAAAGAC  
 GGTGAGCTGGTGTATGGGATAGTGTTCAACCTTGTTACACCGTTTTCCATGAGCAAAC  
 GAAACGTTTTTCATCGCTCTGGAGTGAATACCACGACGATTTCCGGCAGTTTCTACACATA  
 TATTCGCAAGATGTGGCGTGTTACGGTGAAAACCTGGCCTATTTCCCTAAAGGGTTTATT  
 GAGAATATGTTTTCTGCTCTCAGCCAATCCCTGGGTGAGTTTACCAGTTTGTGATTTAAAC  
 GTGGCCAATATGGACAACCTCTTCGCCCCCGTTTTTCACCATGGGCAAAATATTATACGCAA  
 GGCGACAAGGTGCTGATGCCGCTGGCGATTTCAGGTTTCATCATGCCGTCTGTGATGGCTTC  
 CATGTCGGCAGAATGCTTAATGAATTACAACAGTACTGCGATGAGTGGCAGGGCGGGGCG  
 TAAACGCGTGGATCCGGCTTACTAAAAGCCAGATAACAGTATGCGTATTTGCGCGCTGAT  
 TTTTGCGGTATAAGAATATATACTGATATGTATACCCGAAGTATGTCAAAAAGAGGTGTG  
 CTATGAAGCAGCGTATTACAGTGACAGTTGACAGCGACAGCTATCAGTTGCTCAAGGCAT  
 ATATGATGTCAATATCTCCGGTCTGGTAAGCACAACCATGCAGAATGAAGCCCGTCTGCT  
 GCGTGCCGAACGCTGGAAAGCGGAAAATCAGGAAGGGATGGCTGAGGTGCGCCCGGTTTTAT  
 TGAAATGAACGGCTCTTTTGCTGACGAGAACAGGGACTGGTGAAATGCAGTTTAAGGTTT  
 ACACCTATAAAAGAGAGAGCCGTTATCGTCTGTTTGTGGATGTACAGAGTGATATTATTG  
 ACACGCCCCGGGCGACGGATGGTGTATCCCCCTGGCCAGTGACAGTCTGCTGTGAGATAAAG  
 TCTCCCGTGAACCTTTACCCGGTGGTGCATATCGGGGATGAAAGCTGGCGCATGATGACCA  
 CCGATATGGCCAGTGTGCCGGTCTCCGTTATCGGGGAAGAAAGTGCTGATCTCAGCCACC  
 GCGAAAATGACATCAAAAACGCCATTAACTGATGTTCTGGGGAATATAAATGTCAAGGCT  
 CCCTTATACACAGCCAGTCTGCAGGTGACCATAGTGAAGTGGATATGTTGTGTTTTACAG  
 TATTATGTAGTCTGTTTTTTATGCAAAATCTAATTTAATATATTGATATTTATATCATT  
 TACGTTTCTCGTTCAGCTTTCTGTACAAAGTGGTGATTATGTCCCCTATACTAGGTTAT  
 TGGAAAATTAAGGGCCTTGTGCAACCCACTCGACTTCTTTTGAATATCTTGAAGAAAAA  
 TATGAAGAGCATTTGTATGAGCGCGATGAAGGTGATAAATGGCGAAACAAAAAGTTTGAA  
 TTGGGTTTGGAGTTTCCCAATCTTCCTTATTATATTGATGGTGTATGTTAAATTAACACAG  
 TCTATGGCCATCATACGTTATATAGCTGACAAGCACAACATGTTGGGTGGTTGTCCAAAA  
 GAGCGTGCAGAGATTTCAATGCTTGAAGGAGCGGTTTTGGATATTAGATACGGTGTTCG  
 AGAATTGCATATAGTAAAGACTTTGAAACTCTCAAAGTTGATTTTCTTAGCAAGCTACCT  
 GAAATGCTGAAAATGTTTGAAGATCGTTTATGTATATAAACATATTTAAATGGTGATCAT  
 GTAACCCATCCTGACTTCATGTTGTATGACGCTCTTGATGTTGTTTTATACATGGACCCA  
 ATGTGCCCTGGATGCGTTCCCAAAATTAGTTTGTTTTAAAAAACGTATTGAAGCTATCCCA  
 CAAATTGATAAGTACTTGAATCCAGCAAGTATATAGCATGGCCTTTGCAGGGCTGGCAA  
 GCCACGTTTGGTGGTGGCGACCATCTCCAAAATCGGATCTGGTTCCGCGTCCATGGGGA  
 TCCGCTGCTTAACAAAGCCCCGAAAGGAGCTGAGTTGGCTGCTGCCACCGCTGAGCGCTT  
 CCCGATAAGGGAGCAGGCCAGTAAAGCATTACCCGTGGTGGGTTCCCGAGCGGCCAAA  
 GGGAGCAGACTCTAAATCTGCCGTATCGACTTCGAAGGTTTGAATCCTTCCCCACCAC  
 CATCACTTTCAAAGTGAATTGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAA-

FIGURE 96B

ACGGGTCTTGAGGGGTTTTTGTGCTGAAAGGAGGAACATATCCGGATATCCACAGGACGG  
GTGTGGTCGCCATGATCGCGTAGTCGATAGTGGCTCCAAGTAGCGAAGCGAGCAGGACTG  
GGCGGCGGCCAAAGCGGTCCGACAGTGCTCCGAGAACGGGTGCGCATAGAAATTGCATCA  
ACGCATATAGCGCTAGCAGCACGCCATAGTACTGGCGATGCTGTCGGAATGGACGATAT  
CCCGCAAGAGGCCCCGGCAGTACCGGCATAACCAAGCCTATGCCTACAGCATCCAGGGTGA  
CGGTGCCGAGGATGACGATGAGCGCATTGTTAGATTTATACACGGTGCCTGACTGCGTT  
AGCAATTTAACTGTGATAAACTACCGCATTAAGCTTATCGATGATAAGCTGTCAAACAT  
GAGAATTCTTGAAGACGAAAGGGCCTCGTGATACGCCTATTTTATAGGTTAATGTCATG  
ATAATAATGGTTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCT  
ATTTGTTTTATTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCCTGA  
TAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCACATTTCCGTGTGCC  
CTTATTTCCCTTTTTTGCGGCATTTTGCCCTTCTGTTTTTGTCTCACCAGAAACGCTGGTG  
AAAGTAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTC  
AACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAAGCTTTTCCAATGATGAGCACT  
TTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTGTGACGCGGGCAAGAGCAACTC  
GGTCGCGGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAG  
CATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGAT  
AACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTT  
TTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAA  
GCCATACCAAACGACGAGCGTGACACCAGATGCCTGCAGCAATGGCAACAACGTTGCGC  
AAACTATTAAGTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATG  
GAGGCGGATAAAGTTGACAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATT  
GCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTCAGCACTGGGGCCA  
GATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGAT  
GAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCA  
GACCAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGG  
ATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAATCCCTTAACGTGAGTTTTCG  
TTCCACTGAGCGTCAGACCCCGTAGAAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTT  
CTGCGCGTAATCTGCTGCTTGCAAACAAAAAACCCACGCTACCAGCGGTGGTTTGTGTTG  
CCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACCTGGCTTCAGCAGAGCGCAGATA  
CCAAATACTGTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCA  
CCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAG  
TCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCCGGC  
TGAACGGGGGGTTCTGTGCACACAGCCAGCTTGGAGCGAACGACCTACACCGAATGAGA  
TACCATCAGCGTGAGCTATGAGAAAGCGCCACGCTTCCGAAGGGAGAAAGGCGGACAGG  
TATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAAC  
GCCTGGTATCTTTATAGTCCTGTGCGGTTTTCGCCACCTCTGACTTGAGCGTCGATTTTTG  
TGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCCTTTTACGG  
TTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCTGCGTTATCCCTGATTCT  
GTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGAGCCGAACGACC  
GAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCTGATGCGGTATTTTCTCCTT  
ACGCATCTGTGCGGTATTTACACCCGCATATGTTGTCACCTCTCAGTACAATCTGCTCTG  
ATGCCGCATAGTTAAGCCAGTATACACTCCGCTATCGCTACGTGACTGGGTCTATGGCTGC  
GCCCCGACACCCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGGCATC  
CGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTGAGAGGTTTTACCGTC  
ATCACCGAAACGCGCGAGGCAGCTGCGGTAAAGCTCATCAGCGTGGTCTGTAAGCGATTCT  
ACAGATGTCTGCCTGTTTATCCGCGTCCAGCTCGTTGAGTTTCTCCAGAAGCGTTAATGT  
CTGGCTTCTGATAAAGCGGGCCATGTTAAGGGCGGTTTTTCTGTTTGGTCACTGATGC  
CTCCGTGTAAGGGGGATTTCTGTTTATGGGGTAATGATACCGATGAAACGAGAGAGGAT  
GCTCACGATACGGGTACTGATGATGAACATGCCCGGTTACTGGAACGTTGTGAGGGTAA  
ACAACCTGGCGGTATGGATGCGGCGGGACCAGAGAAAAATCACTCAGGGTCAATGCCAGCG  
CTTCGTTAATACAGATGTAGGTGTTCCACAGGGTAGCCAGCAGCATCCTGCGATGCAGAT  
CCGGAACATAATGGTGACGGGCGTGACTTCCGCGTTTCCAGACTTTACGAAACACGGAA  
ACCGAAGACCATTCATGTTGTTGCTCAGGTGCGAGACGTTTTGACAGCAGCAGTCGCTTCA  
CGTTCGCTCGCGTATCGGTGATTCATTCTGCTAACCAAGTAAGGCAACCCCGCAGCCTAG  
CCGGGTCTCAACGACAGGAGCACGATCATGCGCACCCGTGGCCAGGACCCACGCTGCC  
CGAGATGCGCCGCTGCGGCTGCTGGAGATGGCGGACGCGATGGATATGTTCTGCCAAGG  
GTTGGTTTTGCGCATTCACAGTTCTCCGCAAGAATTGATTGGCTCCAATTCTTGGAGTGGT-

FIGURE 96C

229/240

GAATCCGTTAGCGAGGTGCCGCCGGCTTCATTTCAGGTGAGGTGGCCCCGGCTCCATGCA  
CCGCGACGCAACGCGGGGAGGCAGACAAGGTATAGGGCGGCGCCTACAATCCATGCCAAC  
CCGTTCCATGTGCTCGCCGAGGCGGCATAAATCGCCGTGACGATCAGCGGTCCAGTGATC  
GAAGTTAGGCTGGTAAGAGCCGCGAGCGATCCTTGAAGCTGTCCCTGATGGTCGTCATCT  
ACCTGCCTGGACAGCATGGCCTGCAACGCGGGCATCCCGATGCCGCCGGAAGCGAGAAGA  
ATCATAATGGGGAAGGCCATCCAGCCTCGCGTCGCGAACGCCAGCAAGACGTAGCCCAGC  
GCGTCGGCCGCCATGCCGGCGATAATGGCCTGCTTCTCGCCGAAACGTTTGTTGGCGGGA  
CCAGTGACGAAGGCTTGAGCGAGGGCGTGCAAGATTCCGAATACCGCAAGCGACAGGCCG  
ATCATCGTCGCGCTCCAGCGAAAGCGGTCTCGCCGAAAATGACCCAGAGCGCTGCCGGC  
ACCTGTCCTACGAGTTGCATGATAAAGAAGACAGTCATAAGTGCGGCGACGATAGTCATG  
CCCCGCGCCCACCGGAAGGAGCTGACTGGGTTGAAGGCTCTCAAGGGCATCGGTGATCG  
ACGCTCTCCCTTATGCGACTCCTGCATTAGGAAGCAGCCCAGTAGTAGGTTGAGGCCGTT  
GAGCACCGCCGCGCAAGGAATGGTGCATGCAAGGAGATGGCGCCCAACAGTCCCCCGGC  
CACGGGGCCTGCCACCATACCACGCCGAAACAAGCGCTCATGAGCCCGAAGTGGCGAGC  
CCGATCTTCCCCATCGGTGATGTCGGCGATATAGGCGCCAGCAACCGCACCTGTGGCGCC  
GGTGATGCCGGCCACGATGCGTCCGGCGTAGAGG

FIGURE 96D

230/240

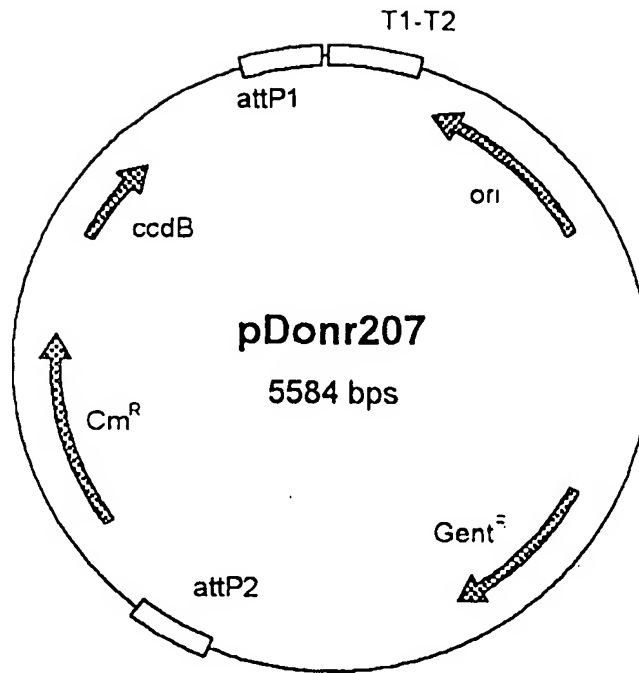


FIGURE 97A

pDONR207 5584 bp

CGGAGAGTAGGGAAGTCCAGGCATCAAATAAAACGAAAGGCTCAGTCGGAAGACTGGGC  
CTTTCGTTTTATCTGTTGTTTGTGCGGTGAACGCTCTCCTGAGTAGGACAAATCCGCCGGG  
AGCGGATTTGAACGTTGTGAAGCAACGGCCCGGAGGGTGGCGGGCAGGACGCCCCGCCATA  
AACTGCCAGGCATCAAATAAGCAGAAGGCCATCCTGACGGATGGCCTTTTTGCGTTTCT  
ACAAACTCTTCCTGGCTAGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGA  
AAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTG  
GCGTTTTTTCATAGGCTCCGCCCCCTGACGAGCATCAAAAAATCGACGCTCAAGTCAG  
AGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTC  
GTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCTTTCTCCCTTCG  
GGAAGCGTGGCGCTTTCTCATAGCTCAGCTGTAGGTATCTCAGTTCGGTGTAGGTGCTT  
CGCTCCAAGCTGGGCTGTGTGCAGCAACCCCGTTACGCCGACCGCTGCGCCTTATCC  
GGTAACATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCC  
ACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGG  
TGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCA  
GTTACCTTCGGAATAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGC  
GGTGGTTTTTTTTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGAT  
CCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAACTCACGTTAAGGGATT  
TTGGTCAATGAGCTTGCGCCGTCCCGTCAAGTCAGCGTAATGCTCTGCCAGTGTTACAACC  
AATTAACCAATTCTGATTAGAAAACTCATCGAGCATCAAATGAACTGCAATTTATTCA  
TATCAGGATTATCAATACCATATTTTTGAAAAAGCCGTTTCTGTAATGAAGGAGAAAACT  
CACCGAGGCAGTTCATAGGATGGCAAGATCCTGGTATCGGTCTGCGATTCCGACTCGTC  
CAACATCAATACAACCTATTAGTAGCCAACCACTAGAACTATAGCTAGAGTCTGGGCGA  
ACAAACGATGCTCGCTTCCAGAAAACCGAGGATGCGAACCCTTCATCCGGGGTCAGCA  
CCACCGGCAAGCGCCGCGACGGCCGAGGTCTTCCGATCTCCTGAAGCCAGGGCAGATCCG  
TGCACAGCACCTTGCCGTAGAAGAACAGCAAGGCCGCAATGCCTGACGATGCGGTGAGA  
CCGAAACCTTGCGCTCGTTCCGCCAGCCAGGACAGAAATGCCTCGACTTCGCTGCTGCCCCA  
AGGTTGCCGGGTGACGCACACCGTGGAACCGATGAAGGCACGAACCCAGTTGACATAAG  
CCTGTTCCGTTCTGTAACTGTAATGCAAGTAGCGTATGCGCTCACGCAACTGGTCCAGAA  
CCTTGACCGAACGCAGCGGTGGTAACGGCGCAGTGGCGGTTTTATGGCTTGTTATGACT  
GTTTTTTTGTACAGTCTATGCCTCGGGCATCCAAGCAGCAAGCGCGTTACGCCGTGGGT  
GATGTTTGATGTTATGGAGCAGCAACGATGTTACGCAGCAGCAACGATGTTACGCAGCAG  
GGCAGTCGCCCTAAAAACAAAGTTAGGTGGCTCAAGTATGGGCATCATTTCGCACATGTAGG  
CTCGGCCCTGACCAAGTCAAATCCATGCGGGCTGCTCTTGATCTTTTCGGTCTGAGTTC  
GGAGACGTAGCCACCTACTCCCAACATCAGCCGACTCCGATTACCTCGGGAACCTTGCTC  
CGTAGTAAGACATTTCATCGCGCTTGCTGCCCTCGACCAAGAAGCGGTTGTTGGCGCTCTC  
GCGGCTTACGTTCTGCCAGGTTTGAGCAGCCGCGTAGTGAGATCTATATCTATGATCTC  
GCAGTCTCCGGCGAGCACCGGAGGCAGGGCATTGCCACCGCGCTCATCAATCTCCTCAAG  
CATGAGGCCAACGCGCTTGCTGCTTATGTGATCTACGTGCAAGCAGATTACGGTGACGAT  
CCCGCAGTGGCTCTCTATACAAAGTTGGGCATACGGGAAGAAGTGATGCACTTTGATATC  
GACCCAAGTACCGCCACCTAACCAATTCGTTCAAGCCGAGATCGGCTTCCCGGCCTAATTT  
CCCCCTCGTCAAAAATAAGGTTATCAAGTGAGAAATCACCATGAGTGACGACTGAATCCGG  
TGAGAATGGCAAAAGTTTATGCATTTCTTTCCAGACTTGTTCAACAGGCCAGCCATTACG  
CTCGTCATCAAATCACTCGCATCAACCAACCGTTATTTCATTTCGTGATTGCGCCTGAGC  
GAGACGAAATACGCGATCGCTGTTAAAAGGACAATTACAAACAGGAATCGAATGCAACCG  
GCGCAGGAACACTGCCAGCGCATCAACAATATTTTACCTGAATCAGGATATTCTTCTAA  
TACCTGGAATGCTGTTTTTCCGGGGATCGCAGTGGTGAGTAACCATGCATCATCAGGAGT  
ACGGATAAAATGCTTGATGGTCGGAAGAGGCATAAATTCCGTACGCCAGTTTAGTCTGAC  
CATCTCATCTGTAAACATCATTTGGCAACGCTACCTTTGCCATGTTTCAGAAACAACTCTGG  
CGCATCGGGCTTCCCATACAAGCGATAGATTGTGCGACCTGATTGCCCGACATTATCGCG  
AGCCCATTTATACCCATATAAATCAGCATCCATGTTGGAATTTAATCGCGGCCTCGACGT  
TTCCCGTTGAATATGGCTCATAACACCCCTGTATTACTGTTTATGTAAGCAGACAGTTT  
TATTGTTTCATGATGATATATTTTTATCTTGTCGAATGTAACATCAGAGATTTTGAGACAC  
GGCCAGAGCTGCAGCTGGATGGCAATAATGATTTTATTTTACTGATAGTGACCTGTT  
CGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGCTG  
AACGAGAAACGTAATAATGATATAAATATCAATATATTAAATTAGATTTTGCATAAAAAAC  
AGACTACATAATACTGTAAAACACAACATATCCAGTCACTATGAATCAACTACTTAGATG-

figure 97B

GTATTAGTGACCTGTAGTCGACTAAGTTGGCAGCATCACCCGACGCACTTTGCGCCGAAT  
AAATACCTGTGACGGAAGATCACTTCGCAGAATAAAATAAATCCTGGTGTCCCTGTTGATA  
CCGGGAAGCCCTGGGCCAACTTTGGCGAAAATGAGACGTTGATCGGCACGTAAGAGGTTT  
CAACTTTCACCATAATGAAATAAGATCACTACCGGGCGTATTTTTTGAGTTATCGAGATT  
TTCAGGAGCTAAGGAAGCTAAAATGGAGAAAAAATCACTGGATATACCACCGTTGATAT  
ATCCCAATGGCATCGTAAAGAACATTTTGAGGCATTTTCAGTCAGTTGCTCAATGTACCTA  
TAACCAGACCGTTTCAGCTGGATATTACGGCCCTTTTTAAAGACCGTAAAGAAAAATAAGCA  
CAAGTTTTATCCGGCCTTTATTACATTCCTTGCCCGCCTGATGAATGCTCATCCGGAATT  
CCGTATGGCAATGAAAGACGGTGAGCTGGTGATATGGGATAGTGTTTACCCCTTGTTACAC  
CGTTTTCCATGAGCAAACCTGAAACGTTTTTCATCGCTCTGGAGTGAATACCACGACGATTT  
CCGGCAGTTTCTACACATATATTGCAAGATGTGGCGTGTTACGGTGAAAACCTGGCCTA  
TTTCCCTAAAGGGTTTATTGAGAATATGTTTTTCGTCTCAGCCAATCCCTGGGTGAGTTT  
CACCACTTTTGATTTAAACGTGGCCAATATGGACAACCTTCTTCGCCCCCGTTTTACCAT  
GGGCAAATATTATACGCAAGGCGACAAGGTGCTGATGCCGCTGGCGATTCAGGTTTCATCA  
TGCCGTCTGTGATGGCTTCCATGTGCGGCAGAATGCTTAATGAATTACAACAGTACTGCGA  
TGAGTGGCAGGGCGGGCGTAATCGCGTGGATCCGGCTTACTAAAAGCCAGATAACAGTA  
TGCGTATTTGCGCGCTGATTTTTGCGGTATAAGAATATATACTGATATGTATACCCGAAG  
TATGTCAAAAAGAGGTGTGCTATGAAGCAGCGTATTACAGTGACAGTTGACAGCGACAGC  
TATCAGTTGCTCAAGGCATATATGATGTCAATATCTCCGGTCTGGTAAGCACAACCATGC  
AGAATGAAGCCCGTCGTCTGCGTGCCGAACGCTGGAAGCGGAAAATCAGGAAGGGATGG  
CTGAGGTCGCCCCGTTTATTGAAATGAACGGCTCTTTTGCTGACGAGAACAGGGACTGGT  
GAAATGCAGTTTAAAGGTTTACACCTATAAAAGAGAGAGCCGTTATCGTCTGTTTGTGGAT  
GTACAGAGTGATATTATTGACACGCCCGGGCGACGGATGGTGATCCCCCTGGCCAGTGCA  
CGTCTGCTGTGAGATAAAGTCTCCCGTGAACCTTTACCCGGTGGTGATATCGGGGATGAA  
AGCTGGCGCATGATGACCACCGATATGGCCAGTGTCGGGTCTCCGTTATCGGGGAAGAA  
GTGGCTGATCTCAGCCACCGCGAAAATGACATCAAAAACGCCATTAAACCTGATGTTCTGG  
GGAATATAAATGTCAGGCTCCCTTATACACAGCCAGTCTGCAGGTCGATACAGTAGAAAT  
TACAGAACTTTATCACGTTTAGTAAGTATAGAGGCTGAAAATCCAGATGAAGCCGAACG  
ACTTGTAAGAGAAAAGTATAAGAGTTGTGAAATTGTTCTTGATGCAGATGATTTTCAGGA  
CTATGACACTAGCGTATATGAATAGGTAGATGTTTTATTTTGTACACAAAAAAGAGGC  
TCGCACCTCTTTTTCTTATTTCTTTTTATGATTTAATACGGCATTGAGGACAATAGCGAG  
TAGGCTGGATACGACGATTCGGTTTGAGAAGAACATTTGGAAGGCTGTGCGTCTGACTAAG  
TTGGCAGCATCACCCGAAGAACATTTGGAAGGCTGTGCGTCTGACTACAGGTCATAATAC  
CATCTAAGTAGTTGATTCATAGTGACTGGATATGTTGTGTTTTACAGTATTATGTAGTCT  
GTTTTTTATGCAAAATCTAATTTAATATATTGATTTTATATCATTTTACGTTTCTCGTT  
CAGCTTTTTTGTACAAAGTTGGCATTATAAAAAAGCATTGCTCATCAATTTGTTGCAACG  
AACAGGTCATATCAGTCAAAATAAATCATTATTTGGGGCCCGAGATCCATGCTAGCGT  
TAAC

FIGURE 97C

# pMAB85

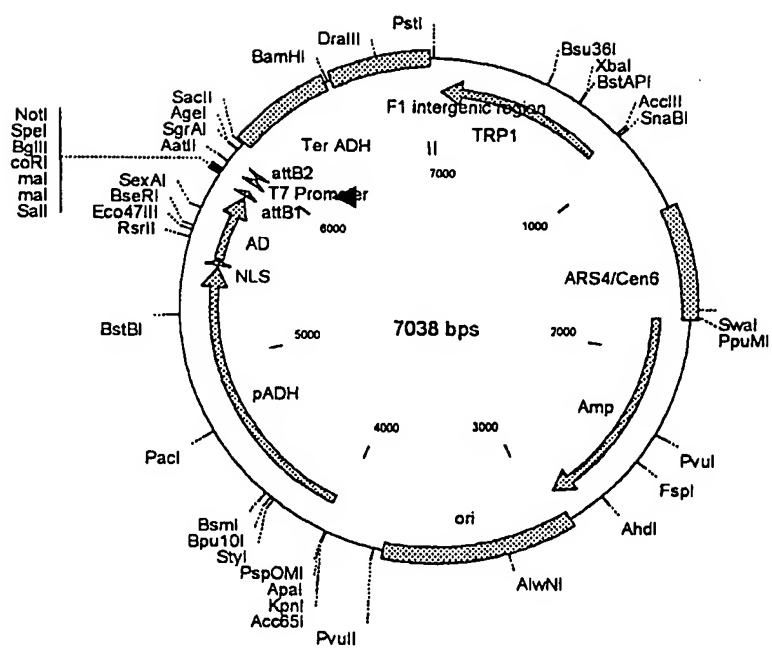


FIGURE 98A

pMAB85 7038 bp

GCCTTACGCATCTGTGCGGTATTTACACCCGAGGCAAGTGCACAAACAATACTTAAATA  
AATACTACTCAGTAATAACCTATTTCTTAGCATTTTGTGACGAAATTTGCTATTTTGTAG  
AGTCTTTTACACCATTTGTCTCCACACCTCCGCTTACATCAACACCAATAACGCCATTTA  
ATCTAAGCGCATCACCAACATTTTCTGGCGTCAGTCCACCAGCTAACATAAAATGTAAGC  
TTTTCGGGGCTCTCTTGCCTTCCAACCCAGTCAGAAATCGAGTTCCAATCCAAAAGTTTAC  
CTGTCCCACTGCTTCTGAATCAAACAAGGGAATAAACGAATGAGGTTTCTGTGAAGCTG  
CACTGAGTAGTATGTTGCAGTCTTTTGAAAATACGAGTCTTTTAATAACTGGCAAACCGA  
GGAACCTCTTGGTATTCTTGGCCAGCACTCATCTCCATGCAGTTGGACGATATCAATGCCGT  
AATCATTGACCAGAGCCAAAACATCCTCCTTAGGTTGATTACGAAACACGCCAACCAAGT  
ATTTCCGAGTGCCTGAACATATTTTATATGCTTTTACAAGACTTGAAATTTTCTTGCAA  
TAACCGGGTCAATTGTTCTCTTTCTATTGGGCACACATATAATACCCAGCAAGTCAGCAT  
CGGAATCTAGAGCACATTCTGCGGCCTCTGTGCTCTGCAAGCCGCAAACCTTTCACCAATG  
GACCAGAACTACCTGTGAAATTAATAACAGACATACTCCAAGCTGCCTTTGTGTGCTTAA  
TCACGTATACTCACGTGCTCAATAGTCACCAATGCCCTCCCTCTTGGCCCTCTCCTTTTC  
TTTTTTTCGACCGAATTAATTCTTAATCGGCCAAAAAAGAAAAGCTCCGGATCAAGATTGT  
ACGTAAGGTGACAAGCTATTTTCAATAAAGAAATATCTTCCACTACTGCCATCTGGCGTC  
ATAACTGCAAAGTACACATATATTACGATGCTGTCTATTAAATGCTTCTATATTATATA  
TATAGTAATGTCGTTTATGGTGCACCTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAA  
GCCAGCCCCGACACCCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGG  
CATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTGAGAGGTTTTCAC  
CGTCATCACCGAAACGCGCGAGACGAAAGGGCCTCGTGATACGCCTATTTTATAGGTTA  
ATGTCATGATAATAATGGTTTCTTAGGACGGATCGCTTGCTGTAACCTACACGCGCCTC  
GTATCTTTTATGATGGAATAATTTGGGAATTTACTCTGTGTTTATTTATTTTATGTTT  
TGATTTGGATTTTAGAAAAGTAAATAAAGAAGGTAGAAGAGTTACGGAATGAAGAAAAA  
AAATAAACAAAGGTTTAAAAAATTTCAACAAAAGCGTACTTTACATATATATTTATTAG  
ACAAGAAAAGCAGATTAAATAGATATACATTGATTAACGATAAGTAAATGTAAATCA  
CAGGATTTTCGTGTGTGGTCTTCTACACAGACAAGATGAAACAATTCGGCATTAATACCT  
GAGAGCAGGAAGAGCAAGATAAAAGGTAGTATTTGTTGGCGATCCCCCTAGAGTCTTTTA  
CATCTTCGGAAAAACAAAACCTATTTTCTTTAATTTCTTTTCTTCTATTTTCTATTTTAA  
TTTATATATTATATTAATAAATTTAAATTAATAATTATTTTATAGCACGTGATGAAAAG  
GACCCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTCTAA  
ATACATTCAAATATGTATCCGCTCATGAGACAATAACCCCTGATAAATGCTTCAATAATAT  
TGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTGCGCCCTTATTCCTTTTTTGCG  
GCATTTTGCCTTCTGTTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAGATGCTGAA  
GATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTT  
GAGAGTTTTCGCCCCGAAGAAGCTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGT  
GGCGCGGTATTATCCCGTATTGACGCGGGCAAGAGCAACTCGGTGCGCGCATACACTAT  
TCTCAGAATGACTTGGTTGAGTACTACCCAGTCACAGAAAAGCATCTTACGGATGGCATG  
ACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTA  
CTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTTCACAACATGGGGGAT  
CATGTAACCTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAG  
CGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACCTATTAACCTGGCGAA  
CTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCA  
GGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCC  
GGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGT  
ATCGTAGTTATCTACACGACGGGCAGTCAGGCAACTATGGATGAACGAAATAGACAGATC  
GCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACGTGTCAGACCAAGTTTACTCATAT  
ATACTTTAGATTGATTTAAACCTTCATTTTTTAATTTAAAGGATCTAGGTGAAGATCCTT  
TTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGAC  
CCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTCTGCGCGTAATCTGCTGC  
TTGCAAAACAAAACCAACCGCTACCAGCGGTGGTTTGTGTTGCGGATCAAGAGCTACCA  
ACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGCTCTTCTA  
GTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCCTACATACCTCGCT  
CTGCTAATCCTGTTACAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTG  
GACTCAAGACGATAGTTACCGGATAAGGCGCAGCGTCCGGCTGAACGGGGGTTCTGTGC-

FIGURE 98B



ACACAGCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCAT  
TGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGG  
GTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGGAACGCCTGGTATCTTTATAGT  
CCTGTGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGG  
CCGAGCCTATGGAAAAACGCCAGCAACGCGGCCCTTTTACGGTTCCTGGCCTTTTGCTGG  
CCTTTTGCTCACATGTTCTTTCTGCGTTATCCCTGATTCTGTGGATAACCGTATTACC  
GCCTTTGAGTGAGCTGATACCGCTCGCCGCGAGCCGAACGACCGAGCGCAGCGAGTCAGTG  
AGCGAGGAAGCGGAAGAGCGCCCAATACGCAAAACCGCCTCTCCCGCGCGTTGGCCGATT  
CATTAAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCA  
ATTAATGTGAGTTACCTCACTCATTAGGCACCCAGGCTTTACACTTTATGCTTCCGGCT  
CCTATGTTGTGTGGAATTGTGAGCGGATAACAATTTACACAGGAAACAGCTATGACCAT  
GATTACGCCAAGCTCGGAATTAACCCCTCACTAAAGGGAACAAAAGCTGGGTACCGGGCCC  
CCCCTCGAGATCCGGGATCGAAGAAATGATGGTAAATGAAATAGGAAATCAAGGAGCATG  
AAGGCAAAAGACAAATATAAGGGTCAACGAAAAATAAAGTGAAAAGTGTGATATGATG  
TATTTGGCTTTGCGGCGCCGAAAAACGAGTTTACGCAATTGCACAATCATGCTGACTCT  
GTGGCGGACCCGCGCTCTTGCCGGCCCGGCGATAACGCTGGGCGTGAGGCTGTGCCCGGC  
GGAGTTTTTTGCGCCTGCATTTTCCAAGGTTTACCCTGCGCTAAGGGGCGAGATTGGAGA  
AGCAATAAGAATGCCGTTGGGGTTGCGATGATGACGACCACGACAACTGGTGTCTATTAT  
TTAAGTTGCCGAAAGAACCTGAGTGCATTTGCAACATGAGTATACTAGAAGAATGAGCCA  
AGACTTGCGAGACGCGAGTTTGCCGGTGGTGCGAACAATAGAGCGACCATGACCTTGAAG  
GTGAGACGCGCATAACCGCTAGAGTACTTTGAAGAGGAAACAGCAATAGGGTTGCTACCA  
GTATAAATAGACAGGTACATAACAACCTGGAATGTTGTCTGTTGAGTACGCTTTCAA  
TTCATTTGGGTGTGCACTTTATTATGTTTACAATATGGAAGGGAACCTTTACACTTCTCCTA  
TGCACATATATTAATTAAAGTCCAATGCTAGTAGAGAAGGGGGGTAAACACCCCTCCGCGC  
TCTTTTCCGATTTTTTTCTAAACCGTGGAATATTTCCGATATCCTTTTGTGTTTCCGGG  
TGTACAATATGGACTTCCTCTTTCTGGCAACCAACCCATACATCGGGATTCTCTATAAT  
ACCTTCGTTGGTCTCCCTAACATGTAGGTGGCGGAGGGGAGATATACAATAGAACAGATA  
CCAGACAAGACATAATGGGCTAAACAAGACTACACCAATTACACTGCCTCATTGATGGTG  
GTACATAACGAACATAACTCTGTAGCCCTGAGAAATGGTTGTCTGTTGAGTACGCTTTCAA  
ACTACCTTTTTTCCATTTGCCATCTATTGAAGTAATAATAGGCGCATGCAACTTCTTTTC  
TTTTTTTTTCTTTCTCTCTCCCCGTTGTTGTCTCACCATATCCGCAATGACAAAAAAA  
ATGATGGAAGACACTAAAGGAAAAAATTAACGACAAAGACAGCACCAACAGATGTCGTTG  
TTCCAGAGCTGATGAGGGGTATCTTCGAACACACGAAACTTTTTCTCTCCTTCATTACG  
CACACTACTCTCTAATGAGCAACGGTATACGGCCTTCTCTCCAGTTACTTGAATTTGAAA  
TAAAAAAGTTTGCCGCTTTGCTATCAAGTATAAATAGACCTGCAATTATTAATCTTTTG  
TTCTCTCGTCATTGTTCTCGTTCCCTTTCTTCTGTTTCTTTTCTGCAATATTTTCA  
AGCTATACCAAGCATACAATCAACTCCAAGCTTATGCCAAGAAGAAGCGGAAGGTCTCG  
AGCGGCGCCAATTTTAATCAAAGTGGGAATATTGCTGATAGCTCATTGTCCTTCACTTTC  
ACTAACAGTAGCAACGGTCCGAACCTCATAACAACCTCAAACAAATTCTCAAGCGCTTTCA  
CAACCAATTGCCTCCTCTAACGTTATGATAACTTCATGAATAATGAAATCACGGCTAGT  
AAAATTGATGATGGTAATAATTCAAACCACTGTACCTGGTTGGACGGACCAAACTGCG  
TATAACGCGTTTGAATCACTACAGGGATGTTTAAATACCACTACAATGGATGATGTATAT  
AACTATCTATTTCGATGATGAAGATACCCACCAAAACCAAAAAAGAGGGTGGGTGCGATC  
ACAAGTTTGTACAAAAAAGCAGGCTTGTGACCCCGGGAATTTCAGATCTACTAGTGCGGC  
CGCACGCGTACCCAGCTTTCTTGTACAAAGTGGTGACGTGAGCTCCCTATAGTGAGTCG  
TATTACACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACACCGGTGAGCTCTAAGT  
AAGTAACGGCCCGCCACCGCGGTGGAGCTTTGGACTTCTTCGCCAGAGGTTTGGTCAAGTC  
TCCAATCAAGGTTGTGCGCTTGTCTACCTTGCCAGAAATTTACGAAAAGATGAAAAGGG  
TCAAATCGTTGGTAGATACGTTGTTGACACTTCTAAATAAGCGAATTTCTTATGATTTAT  
GATTTTTATTATTAAATAAGTTATAAAAAAATAAGTGTATACAAATTTTAAAGTGACTC  
TTAGGTTTTTAAACGAAATTTCTTGTCTTGAGTAACTCTTTCCTGTAGGTGAGTTGCT  
TTCTCAGGTATAGCATGAGGTGCTCTTATTGACCACACCTCTACCGGCATGCCGAGCAA  
ATGCCTGCAAAATCGCTCCCATTTTACCCAATTGTAGATATGCTAACTCCAGCAATGAGT  
TGATGAATCTCGGTGTGTATTTTATGTCCTCAGAGGACAATACCTGTTGTAATCGTTCTT  
CCACACGGATCCGCATCAGGCGAAATGTAAACGTTAATATTTTGTAAAAATTCGCGTTA  
AATATTTGTTAAATCAGCTCATTTTTTAAACCAATAGGCCGAAATCGGCAAAATCCCTTAT  
AAATCAAAAGAATAGACCGAGATAGGGTTGAGTGTGTTCCAGTTTGGAAACAAGAGTCCA  
CTATTAAAGAACGTGGACTCCAACGTCAAAGGGCGAAAAACCGTCTATCAGGGCGATGGC-

FIGURE 98C

CCACTACGTGAACCATCACCCCTAATCAAGTTTTTTGGGGTCGAGGTGCCGTAAAGCACTA  
AATCGGAACCCTAAAGGGAGCCCCGATTTAGAGCTTGACGGGGAAAGCCGGCGAACGTG  
GCGAGAAAGGAAGGGAAGAAAGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCG  
GTCACGCTGCGCGTAACCACCACACCCGCCGCGCTTAATGCGCCGCTACAGGGCGCGTCC  
CATTCGCCATTCACTGCA

FIGURE 98D

# pMAB86

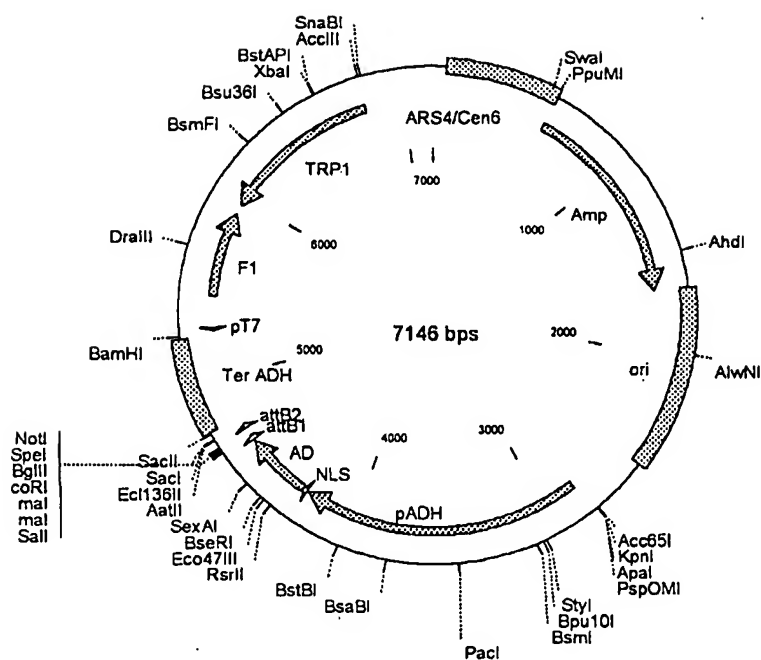


FIGURE 99A

pMAB86

7146 bp

GACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTCATGATAATAATGGTTT  
CTTAGGACGGATCGCTTGCCTGTAACCTACACGCGCCTCGTATCTTTAATGATGGAATA  
ATTTGGGAATTTACTCTGTGTTTATTTATTTTTATGTTTTGTATTTGGATTTTAGAAAGT  
AAATAAGAAGGTAGAAGAGTTACGGAATGAAGAAAAAATAAACAAAGGTTTAAAAA  
ATTTCAACAAAAAGCGTACTTTACATATATATTTATTAGACAAGAAAAAGCAGATTAAATA  
GATATACATTCGATTAACGATAAGTAAAATGTAAAATCACAGGATTTTCGTGTGTGGTCT  
TCTACACAGACAAGATGAAACAATTTCGGCATTAAATACCTGAGAGCAGGAAGAGCAAGATA  
AAAGGTAGTATTTGTTGGCGATCCCCCTAGAGTCTTTTACATCTTCGGAAAACAAAACT  
ATTTTTTCTTTAATTTCTTTTTTTTACTTTCTATTTTTTAATTTATATATTTATATTAAAAA  
ATTTAAATTATAATTATTTTTTATAGCACGTGATGAAAAGGACCCAGGTGGCACTTTTCGG  
GGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCG  
CTCATGAGACAATAAACCTGATAAATGCTTCAATAATATTGAAAAAGGAAGATGAGT  
ATTCAACATTTCCGTGTGCGCCCTTATCCCTTTTTTTCGGCATTTCCTTCTGTTTTT  
GCTCACCCAGAAACGCTGGTGAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTG  
GGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAA  
CGTTTTTCCAATGATGAGCACTTTTAAAGTTCGTATGTGGCGCGGTATTATCCCGTATT  
GACGCCGGGCAAGAGCAACTCGGTCCGCCGATACACTATTCTCAGAATGACTTGGTTGAG  
TACTCACCACTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCGAT  
GCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGA  
CCGAAGGAGCTAACCGCTTTTTTTCACAACATGGGGGATCATGTAACTCGCCTTGATCGT  
TGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCAGATGCCTGTA  
GCAATGGCAACAACGTTGCGCAAACTATTAACCTGGCGAACTACTTACTCTAGCTTCCCGG  
CAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCC  
CTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGT  
ATCCTTGACGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACG  
GGCAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTG  
ATTAAGCATTTGGTAACTGTGACCAAGTTTACTCATATATACTTTAGATTGATTTAAAA  
CTTCATTTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAA  
ATCCCTTAACGTGAGTTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGA  
TCTTCTTGAGATCCTTTTTTCTGCGCGTAATCTGCTGCTTGCAAAACAAAAAACACCG  
CTACCAGCGGTGGTTTGTGTGCGGATCAAGAGCTACCAACTCTTTTCCGAAGGTAAC  
GGCTTCAGCAGAGCGCAGATACCAAATACTGTCTTCTAGTGTAAGCGTAGTTAGGCCAC  
CACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTG  
GCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCG  
GATAAGGCGCAGCGGTGCGGCTGAACGGGGGGTTTCGTGCACACAGCCCAGCTTGGAGCGA  
ACGACCTACACCGAACTGAGATACCTACAGCGTGAGCATTGAGAAAAGCGCCACGCTTCCC  
GAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTTCGGAACAGGAGAGCGCACG  
AGGGAGCTTCCAGGGGGGAACGCCTGGTATCTTTATAGTCCGTGTCGGGTTTCGCCACCTC  
TGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGAGCCTATGGAAAAACGCC  
AGCAACGCGGCCTTTTTACGGTTCCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTT  
CCTGCGTTATCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACC  
GCTCGCCGACGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGC  
CCAATACGCAACCGCCTCTCCCCGCGCGTTGGCCGATTCAATATGCAGCTGGCACGAC  
AGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTACCTCACT  
CATTAGGCACCCAGGCTTTACACTTTATGCTTCCGGCTCCTATGTTGTGTGGAATTGTG  
AGCGGATAACAATTTACACAGGAAACAGCTATGACCATGATTACGCCAAGCTCGGAATT  
AACCCTCACTAAAGGGAACAAAAGCTGGGTACCGGGCCCCCTCGAGATCCGGGATCGA  
AGAAATGATGGTAAATGAAATAGGAAATCAAGGAGCATGAAGGCAAAAGACAAATATAAG  
GGTCAACGAAAAATAAAGTGAAGAGTGTGATATGATGATTTGGCTTTGCGGCGCCGA  
AAAAACGAGTTTACGCAATTGCACAATCATGCTGACTCTGTGGCGGACCCGCGCTCTTGC  
CGCCCGGCGGATAACGCTGGCGTGAGGCTGTGCCCGGCGGAGTTTTTTGCGCCTGCATT  
TTCCAAGGTTTACCCTGCGCTAAGGGGCGAGATTGGAGAAGCAATAAGAATGCCGGTTGG  
GGTTGCGATGATGACGACCACGACAACCTGGTGTCAATTATTAAGTTGCCGAAAGAACCTG  
AGTGCAATTTGCAACATGAGTATACTAGAAGAATGAGCCAAGACTTGCAGACGCGAGTTT  
GCCGGTGGTGCAACAATAGAGCGACCATGACCTTGAAGGTGAGACGCGCATAACCGCTA-

FIGURE 99B

GAGTACTTTGAAGAGGAAACAGCAATAGGGTTGCTACCAGTATAAATAGACAGGTACATA  
CAACACTGGAAATGGTTGTCTGTTTGAGTACGCTTTCAATTCATTTGGGTGTGCACTTTA  
TTATGTTACAATATGGAAGGGAACCTTACACTTCTCCTATGCACATATATTAATTAAAGT  
CCAATGCTAGTAGAGAAGGGGGTAACACCCCTCCGCGCTCTTTCCGATTTTTTTCTAA  
ACCGTGGAAATATTTTCGGATATCCTTTTGTGTTTCCGGGTGTACAATATGGACTTCCTCT  
TTTCTGGCAACCAAACCCATACATCGGGATTCTATAATACCTTCGTTGGTCTCCCTAAC  
ATGTAGGTGGCGGAGGGGAGATATACAATAGAACAGATACCAGACAAGACATAATGGGCT  
AAACAAGACTACACCAATTACACTGCCTCATTGATGGTGTTACATAACGAACATAACTG  
TAGCCCTAGACTTGTATAGCCATCATCATATCGAAGTTTCACTACCCTTTTTCCATTTGCC  
ATCTATTGAAGTAATAATAGGCGCATGCAACTTCTTTCTTTTTTTTTCTTTCTCTCTC  
CCCCGTTGTTGTCTCACCATATCCGCAATGACAAAAAATGATGGAAGACACTAAAGGA  
AAAAATTAACGACAAAGACAGCACCAACAGATGTCGTTGTTCCAGAGCTGATGAGGGGTA  
TCTTCGAACACACGAAACTTTTTCTTCTCTTCATTACGCACTACTCTCTAATGAGCA  
ACGGTATACGGCCTTCCTTCCAGTTACTTGAATTTGAAATAAAAAAGTTTGCCGCTTG  
CTATCAAGTATAAAATAGACCTGCAATTATTAATCTTTGTTTCTCCTCGTCATTGTCGT  
TCCCTTTCTTCTTGTCTTTCTTTTCTGCACAATATTTCAAGCTATACCAAGCATACAATC  
AACTCCAAGCTTATGCCCAAGAAGAAGCGGAAGGTCTCGAGCGGCGCAATTTTAATCAA  
AGTGGGAATATTGCTGATAGCTCATTGTCTTCACTTTCACATAACAGTAGCAACGGTCCG  
AACCTCATAACAACCTCAAACAAATTCTCAAGCGCTTTCACAACCAATTGCCTCCTCTAAC  
GTTTCATGATAACTTCATGAATAATGAAATCACGGCTAGTAAATTTGATGATGGTAATAAT  
TCAAAACCACCTGTCACCTGGTTGGACGGACCAAACTGCGTATAACGCGTTTGGTAACACT  
ACAGGGATGTTTAAATACCACTACAATGGATGATGTATATAACTATCTATTGATGATGAA  
GATACCCACCAAACCCAAAAAAGAGGGTGGGTCGATCACAAGTTTGTACAAAAAAGCA  
GGCTTGTGACCCCGGAATTGAGATCTACTAGTGCGGCCGCACGCGTACCCAGCTTTCT  
TGTACAAAGTGGTGACGTCGAGCTCTAAGTAAGTAACGGCCGCCACCGCGGTGGAGCTTT  
GGACTTCTTCGCCAGAGGTTTGGTCAAGTCTCCAATCAAGGTTGTGCGGCTTGTCTACCTT  
GCCAGAAATTTACGAAAAGATGGAAGGGTCAAATCGTTGGTAGATACGTTGTTGACAC  
TTCATAAATAGCGAATTTCTTATGATTTATGATTTTATTATTAAATAAGTTATAAAAAA  
AATAAGTGATACAAATTTTAAAGTGACTCTTAGGTTTAAAACGAAAATTTCTGTTCTT  
GAGTAACTCTTTCCTGTAGGTGAGGTGCTTCTCAGGTATAGCATGAGGTGCTCTTAT  
TGACCACACCTCTACCGGCATGCCGAGCAAATGCCTGCAAATCGCTCCCCATTTACCCA  
ATTGTAGATATGCTAACTCCAGCAATGAGTTGATGAATCTCGGTGTGATTTTATGTCCT  
CAGAGGACAATACCTGTTGTAATCGTTCTTCCACACGGATCCCAATTGCCCCATAGTGA  
GTCGTATTACAATTCCTGGCCGTGCTTTTACAACGTCGTGACTGGGAAAAACCTTGGCGT  
TACCAAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGA  
GGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGACGCGCCC  
TGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTT  
GCCAGCGCCCTAGCGCCGCTCCTTTCGCTTTCTTCCCTTCTTTCTCGCCACGTTTCGCC  
GGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTA  
CGGCACCTCGACCCCAAAAACTTGATTAGGGTGATGGTTACGTTAGTGGGCCATCGCCC  
TGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTG  
TTCCAAACTGGAACAACACTCAACCTATCTCGGTCTATTCTTTTGATTTATAAGGGATT  
TTGCCGATTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACGCGAAT  
TTTAACAAAATATTAAACGTTTACAATTTCTTGATGCGGTATTTTCTCCTTACGCATCTGT  
GCGGTATTTACACCGCAGGCAAGTGCACAAACAATACTTAAATAAATACTACTCAGTAA  
TAACCTATTTCTTAGCATTTTTGACGAAATTTGCTATTTTGTAGAGTCTTTTACACCAT  
TTGCTCTCCACACCTCCGCTTACATCAACACCAATAACGCCATTTAATCTAAGCGCATCAC  
CAACATTTTTCTGGGTCAGTCCACAGCTAACATAAAATGTAAGCTTTCGGGGCTCTCTT  
GCCTTCCAACCCAGTCAGAAATCGAGTTCCAATCCAAAAGTTACCTGTCCACCTGCTT  
CTGAATCAAACAAGGGAATAAACGAATGAGGTTTCTGTGAAGCTGCACTGAGTAGTATGT  
TGCAGTCTTTTGGAAATACGAGTCTTTTAATAACTGGCAAACCGAGGAACCTTGGTATT  
CTTGCCACGACTCATCTCCATGCAGTTGGACGATATCAATGCCGTAATCATTGACCAGAG  
CCAAAACATCCTCCTTAGGTTGATTACGAAACACGCCAACCAAGTATTTCCGGAGTGCCTG  
AACTATTTTATATGCTTTTACAAGACTTGAATTTTCTTGAATAACCGGGTCAATTG  
TTCTCTTTCTATTGGGCACACATATAATACCCAGCAAGTCAGCATCGGAATCTAGAGCAC  
ATTCTGCGGCCTCTGTGCTCTGCAAGCCGCAAACTTTACCAATGGACCAGAATACTG  
TGAAATTAATAACAGACATACTCCAAGCTGCCTTTGTGTGCTTAATCACGTATACTCACG  
TGCTCAATAGTCACCAATGCCCTCCCTCTTGGCCCTCTCCTTTCTTTTTTCGACCGAAT-


TAATTCCTTAATCGGCAAAAAAGAAAAGCTCCGGATCAAGATTGTACGTAAGGTGACAAG  
CTATTTTTCAATAAAGAATATCTTCCACTACTGCCATCTGGCGTCATAACTGCAAAGTAC  
ACATATATTACGATGCTGTCTATTAAATGCTTCCTATATTATATATATAGTAATGTCGTT  
TATGGTGCACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGCCCCGACACC  
CGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAGAC  
AAGCTGTGACCGTCTCCGGGAGCTGCATGTGTGTCAGAGGTTTTCACCGTCATCACCGAAAC  
GCGCGA

FIGURE 99D

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM  
(PCT Rule 13bis)


SM  
REC'D

A. The indications made below relate to the microorganism referred to in the description on page <u>54</u> , line <u>8</u> .	
<b>B. IDENTIFICATION OF DEPOSIT</b> <div style="text-align: right;">Further deposits are identified on an additional sheet <input checked="" type="checkbox"/></div>	
Name of depositary institution  Agricultural Research Culture Collection (NRRL) International Depository Authority	
Address of depositary institution (including postal code and country)  1815 N. University Street Peoria, Illinois 61604 United States of America	
Date of deposit    February 27, 1999	Accession Number    NRRL B-30103
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) <div style="text-align: right;">This information is continued on an additional sheet <input type="checkbox"/></div>	
Escherichia coli DB3.1(pEZC15101)  In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indications are not for all designated States)	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")	

For receiving Office use only	For International Bureau use only
<input checked="" type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer 	Authorized officer

**INDICATIONS RELATING TO A DEPOSITED MICROORGANISM**  
(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>55</u> , line <u>16</u> .	
<b>B. IDENTIFICATION OF DEPOSIT</b> <span style="float: right;">Further deposits are identified on an additional sheet <input checked="" type="checkbox"/></span>	
Name of depositary institution  Agricultural Research Culture Collection (NRRL) International Depositary Authority	
Address of depositary institution (including postal code and country)  1815 N. University Street Peoria, Illinois 61604 United States of America	
Date of deposit     February 27, 1999	Accession Number     NRRL B-30100
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
Escherichia coli DB3.1(pENTR-1A)  In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indications are not for all designated States)	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")	

For receiving Office use only	For International Bureau use only
<input checked="" type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer 	Authorized officer



**INDICATIONS RELATING TO A DEPOSITED MICROORGANISM**  
(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>55</u> , line <u>16</u> .	
<b>B. IDENTIFICATION OF DEPOSIT</b> <span style="float: right;">Further deposits are identified on an additional sheet <input checked="" type="checkbox"/></span>	
Name of depositary institution  Agricultural Research Culture Collection (NRRL) International Depository Authority	
Address of depositary institution (including postal code and country)  1815 N. University Street Peoria, Illinois 61604 United States of America	
Date of deposit     February 27, 1999	Accession Number     NRRL B-30102
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
Escherichia coli DB3.1(pENTR-3C)  In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indications are not for all designated States)	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")	

<p style="text-align: center;">For receiving Office use only</p> <div style="border: 1px solid black; padding: 5px; margin-bottom: 5px;"> <input checked="" type="checkbox"/> This sheet was received with the international application         </div> <div style="border: 1px solid black; padding: 5px;">           Authorized officer  </div>	<p style="text-align: center;">For International Bureau use only</p> <div style="border: 1px solid black; padding: 5px; margin-bottom: 5px;"> <input type="checkbox"/> This sheet was received by the International Bureau on:         </div> <div style="border: 1px solid black; padding: 5px; height: 40px;">           Authorized officer         </div>
---	--

**INDICATIONS RELATING TO A DEPOSITED MICROORGANISM**  
(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>55</u> , line <u>16</u> .	
<b>B. IDENTIFICATION OF DEPOSIT</b> <span style="float: right;">Further deposits are identified on an additional sheet <input checked="" type="checkbox"/></span>	
Name of depositary institution  Agricultural Research Culture Collection (NRRL) International Depository Authority	
Address of depositary institution (including postal code and country)  1815 N. University Street Peoria, Illinois 61604 United States of America	
Date of deposit     February 27, 1999	Accession Number     NRRL B-30101
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
Escherichia coli DB3.1(pENTR-2B)  In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indications are not for all designated States)	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")	

<p style="text-align: center;">For receiving Office use only</p> <p><input checked="" type="checkbox"/> This sheet was received with the international application</p> <p>Authorized officer <i>B. Fudai</i></p>	<p style="text-align: center;">For International Bureau use only</p> <p><input type="checkbox"/> This sheet was received by the International Bureau on:</p> <p>Authorized officer</p>
--	--

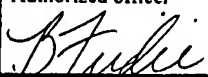
INDICATIONS RELATING TO A DEPOSITED MICROORGANISM  
(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>20-21</u> . <div style="float: right; text-align: right;"> <div style="border: 1px solid black; padding: 2px;">WIPO</div> <div style="border: 1px solid black; padding: 2px;">PCT</div> </div>	
<b>B. IDENTIFICATION OF DEPOSIT</b> <div style="text-align: right;">Further deposits are identified on an additional sheet <input checked="" type="checkbox"/></div>	
Name of depositary institution  Agricultural Research Culture Collection (NRRL) International Depository Authority	
Address of depositary institution (including postal code and country)  1815 N. University Street Peoria, Illinois 61604 United States of America	
Date of deposit    February 27, 1999	Accession Number    NRRL B-30108
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) <div style="text-align: right;">This information is continued on an additional sheet <input type="checkbox"/></div>	
Escherichia coli DB10B(pCMVSPORT6)  In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indications are not for all designated States)	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")	

For receiving Office use only	For International Bureau use only
<input checked="" type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer 	Authorized officer

**INDICATIONS RELATING TO A DEPOSITED MICROORGANISM**  
(PCT Rule 13bis)

<b>A.</b> The indications made below relate to the microorganism referred to in the description on page <u>54</u> , line <u>9</u> .	
<b>B. IDENTIFICATION OF DEPOSIT</b> <span style="float: right;">Further deposits are identified on an additional sheet <input checked="" type="checkbox"/></span>	
Name of depositary institution  Agricultural Research Culture Collection (NRRL) International Depository Authority	
Address of depositary institution (including postal code and country)  1815 N. University Street Peoria, Illinois 61604 United States of America	
Date of deposit    February 27, 1999	Accession Number    NRRL B-30105
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
Escherichia coli DB3.1(pEZC15103)  In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indications are not for all designated States)	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")	

<b>For receiving Office use only</b>	<b>For International Bureau use only</b>
<input checked="" type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer 	Authorized officer

**INDICATIONS RELATING TO A DEPOSITED MICROORGANISM**  
(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>54</u> , line <u>9</u> .	
<b>B. IDENTIFICATION OF DEPOSIT</b> <span style="float: right;">Further deposits are identified on an additional sheet <input checked="" type="checkbox"/></span>	
Name of depositary institution Agricultural Research Culture Collection (NRRL) International Depository Authority	
Address of depositary institution (including postal code and country) 1815 N. University Street Peoria, Illinois 61604 United States of America	
Date of deposit    February 27, 1999	Accession Number    NRRL B-30104
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
Escherichia coli DB3.1(pEZC15102) In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indications are not for all designated States)	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")	

<p style="text-align: center;">For receiving Office use only</p> <div style="border: 1px solid black; padding: 5px; margin-bottom: 5px;"> <input checked="" type="checkbox"/> This sheet was received with the international application         </div> <div style="border: 1px solid black; padding: 5px;">           Authorized officer  </div>	<p style="text-align: center;">For International Bureau use only</p> <div style="border: 1px solid black; padding: 5px; margin-bottom: 5px;"> <input type="checkbox"/> This sheet was received by the International Bureau on:         </div> <div style="border: 1px solid black; padding: 5px;">           Authorized officer         </div>
---	--

**INDICATIONS RELATING TO A DEPOSITED MICROORGANISM**  
(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>52</u> , line <u>31</u> .	
<b>B. IDENTIFICATION OF DEPOSIT</b> <span style="float:right">Further deposits are identified on an additional sheet <input checked="" type="checkbox"/></span>	
Name of depositary institution Agricultural Research Culture Collection (NRRL) International Depository Authority	
Address of depositary institution (including postal code and country)  1815 N. University Street Peoria, Illinois 61604 United States of America	
Date of deposit    February 27, 1999	Accession Number    NRRL B-30099
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) <span style="float:right">This information is continued on an additional sheet <input type="checkbox"/></span>	
Escherichia coli DB3.1(pAHPK <sub>an</sub> ) or Escherichia coli DB3.1(pAttPK <sub>an</sub> )  In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indications are not for all designated States)	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")	

<p align="center">For receiving Office use only</p> <p><input checked="" type="checkbox"/> This sheet was received with the international application</p> <p>Authorized officer    Barbara Fridie PCT Operations - IFPD Team 1 763) 305-3230 (FAX)</p>	<p align="center">For International Bureau use only</p> <p><input type="checkbox"/> This sheet was received by the International Bureau on:</p> <p>Authorized officer</p>
--	---

*Escherichia coli* DB3.1(pENTR-3C)**ICELAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Icelandic Patent Office), or has been finally decided upon by the Icelandic Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in Rule 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

**NORWAY**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Norwegian Patent office or any person approved by the applicant in the individual case.

**SINGAPORE**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

*Escherichia coli* DB3.1(pENTR-3C)

#### SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent office or any person approved by the applicant in the individual case.

#### UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.



*Escherichia coli* DB3.1(pENTR-2B)

## AUSTRALIA

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

## CANADA

The applicant hereby requests that, until either a Canadian patent has been issued on the basis of the application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the furnishing of a sample of deposited biological material referred to in the application only be effected to an independent expert nominated by the Commissioner of Patents.

## DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent office or any person approved by the applicant in the individual case.

## FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Registration), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the National Board of Patents and Registration or any person approved by the applicant in the individual case.

*Escherichia coli* DB3.1(pENTR-2B)

#### ICELAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the Icelandic Patent Office), or has been finally decided upon by the Icelandic Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

#### NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in Rule 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

#### NORWAY

The applicant hereby requests that, until the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Norwegian Patent office or any person approved by the applicant in the individual case.

#### SINGAPORE

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

*Escherichia coli* DB3.1(pENTR-2B)

#### SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent office or any person approved by the applicant in the individual case.

#### UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

*Escherichia coli* DB3.1(pENTR-1A)

## AUSTRALIA

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

## CANADA

The applicant hereby requests that, until either a Canadian patent has been issued on the basis of the application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the furnishing of a sample of deposited biological material referred to in the application only be effected to an independent expert nominated by the Commissioner of Patents.

## DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent office or any person approved by the applicant in the individual case.

## FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Registration), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the National Board of Patents and Registration or any person approved by the applicant in the individual case.

*Escherichia coli* DB3.1(pENTR-1A)**ICELAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Icelandic Patent Office), or has been finally decided upon by the Icelandic Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in Rule 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

**NORWAY**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Norwegian Patent office or any person approved by the applicant in the individual case.

**SINGAPORE**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

*Escherichia coli* DB3.1(pENTR-1A)

#### SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent office or any person approved by the applicant in the individual case.

#### UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

*Escherichia coli DB10B(pCMVSPORT6)*

## AUSTRALIA

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

## CANADA

The applicant hereby requests that, until either a Canadian patent has been issued on the basis of the application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the furnishing of a sample of deposited biological material referred to in the application only be effected to an independent expert nominated by the Commissioner of Patents.

## DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent office or any person approved by the applicant in the individual case.

## FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Registration), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the National Board of Patents and Registration or any person approved by the applicant in the individual case.

*Escherichia coli* DB3.1(pAHPKan) or *Escherichia coli* DB3.1(pAttPKan)

## AUSTRALIA

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

## CANADA

The applicant hereby requests that, until either a Canadian patent has been issued on the basis of the application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the furnishing of a sample of deposited biological material referred to in the application only be effected to an independent expert nominated by the Commissioner of Patents.

## DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent office or any person approved by the applicant in the individual case.

## FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Registration), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the National Board of Patents and Registration or any person approved by the applicant in the individual case.



*Escherichia coli* DB3.1(pAHPKan) or *Escherichia coli* DB3.1(pAttPKan)

#### ICELAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the Icelandic Patent Office), or has been finally decided upon by the Icelandic Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

#### NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in Rule 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

#### NORWAY

The applicant hereby requests that, until the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Norwegian Patent office or any person approved by the applicant in the individual case.

#### SINGAPORE

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

*Escherichia coli* DB3.1(pAHPKan) or *Escherichia coli* DB3.1(pAttPKa~~h~~)

#### SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent office or any person approved by the applicant in the individual case.

#### UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

*Escherichia coli DB10B(pCMVSPORT6)*

#### ICELAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the Icelandic Patent Office), or has been finally decided upon by the Icelandic Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

#### NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in Rule 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

#### NORWAY

The applicant hereby requests that, until the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Norwegian Patent office or any person approved by the applicant in the individual case.

#### SINGAPORE

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

*Escherichia coli DB10B(pCMVSPORT6)*

#### **SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent office or any person approved by the applicant in the individual case.

#### **UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

*Escherichia coli DB3.1(pEZC15103)***AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

**CANADA**

The applicant hereby requests that, until either a Canadian patent has been issued on the basis of the application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the furnishing of a sample of deposited biological material referred to in the application only be effected to an independent expert nominated by the Commissioner of Patents.

**DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent office or any person approved by the applicant in the individual case.

**FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Registration), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the National Board of Patents and Registration or any person approved by the applicant in the individual case.

*Escherichia coli* DB3.1(pEZC15103)

#### ICELAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the Icelandic Patent Office), or has been finally decided upon by the Icelandic Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

#### NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in Rule 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

#### NORWAY

The applicant hereby requests that, until the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Norwegian Patent office or any person approved by the applicant in the individual case.

#### SINGAPORE

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

*Escherichia coli* DB3.1(pEZC15103)

#### SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent office or any person approved by the applicant in the individual case.

#### UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

*Escherichia coli* DB3.1(pEZC15102)**AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

**CANADA**

The applicant hereby requests that, until either a Canadian patent has been issued on the basis of the application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the furnishing of a sample of deposited biological material referred to in the application only be effected to an independent expert nominated by the Commissioner of Patents.

**DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent office or any person approved by the applicant in the individual case.

**FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Registration), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the National Board of Patents and Registration or any person approved by the applicant in the individual case.



*Escherichia coli* DB3.1(pEZC15102)

#### ICELAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the Icelandic Patent Office), or has been finally decided upon by the Icelandic Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

#### NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in Rule 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

#### NORWAY

The applicant hereby requests that, until the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Norwegian Patent office or any person approved by the applicant in the individual case.

#### SINGAPORE

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

*Escherichia coli* DB3.1(pEZC15102)

#### **SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent office or any person approved by the applicant in the individual case.

#### **UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

*Escherichia coli* DB3.1(pEZC15101)

## AUSTRALIA

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

## CANADA

The applicant hereby requests that, until either a Canadian patent has been issued on the basis of the application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the furnishing of a sample of deposited biological material referred to in the application only be effected to an independent expert nominated by the Commissioner of Patents.

## DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent office or any person approved by the applicant in the individual case.

## FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Registration), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the National Board of Patents and Registration or any person approved by the applicant in the individual case.

*Escherichia coli* DB3.1(pEZC15101)

#### ICELAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the Icelandic Patent Office), or has been finally decided upon by the Icelandic Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

#### NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in Rule 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

#### NORWAY

The applicant hereby requests that, until the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Norwegian Patent office or any person approved by the applicant in the individual case.

#### SINGAPORE

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

*Escherichia coli DB3.1(pEZC15101)***SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent office or any person approved by the applicant in the individual case.

**UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

*Escherichia coli DB3.1(pENTR-3C)***AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

**CANADA**

The applicant hereby requests that, until either a Canadian patent has been issued on the basis of the application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the furnishing of a sample of deposited biological material referred to in the application only be effected to an independent expert nominated by the Commissioner of Patents.

**DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent office or any person approved by the applicant in the individual case.

**FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Registration), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the National Board of Patents and Registration or any person approved by the applicant in the individual case.

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US00/05432

<b>A. CLASSIFICATION OF SUBJECT MATTER</b>		
IPC(7) : Please See Extra Sheet.		
US CL : 435/91.2, 252.3, 320.1; 530/350; 536/ 23.1, 24.1		
According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b>		
Minimum documentation searched (classification system followed by classification symbols)		
U.S. : 435/91.2, 252.3, 320.1; 530/350; 536/ 23.1, 24.1		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
NONE		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)		
Please See Extra Sheet.		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P ---- Y,P	US 5,888,732 A (HARTLEY et al.) 30 March 1999, see entire document.	1-21, 25-30 36-38 ----- 22-24, 31-35
X - Y	HASAN et al. Escherichia coli genome targeting, I. Cre-lox-mediated in vitro generation of ori- plasmids and their in vivo chromosomal integration and retrieval. Gene. 1994, Vol. 150, pages 51-56, see entire document.	1-5, 10, 11, 19-21 ----- 15-18, 22-38
X - Y	KATZ et al. Site-specific recombination in Escherichia coli between the att sites of plasmid pSE211 from Saccharopolyspora erythraea. Mol. Gen. Genet. 1991, Vol. 227, pages 155-159, see entire document.	1-11, 19-21 ----- 15-18, 22-38
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E* earlier document published on or after the international filing date	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*G*	document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means		
*P* document published prior to the international filing date but later than the priority date claimed		
Date of the actual completion of the international search	Date of mailing of the international search report	
08 MAY 2000	23 MAY 2000	
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer <i>Christina Lawrence Fox</i> IREM YUCEL	
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196	

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US00/05432

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X - Y	ASTUMIAN et al. Site-specific recombination between cloned attP and attB sites from the Haemophilus influenzae bacteriophage HP1 propagated in recombination deficient Escherichia coli. J of Bacteriology. March 1989, Vol. 171, No. 3, pages 1747-1750, see entire document.	1-11, 19-21 ----- 15-18, 22-38



# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US00/05432

## A. CLASSIFICATION OF SUBJECT MATTER: IPC (7):

C07H 21/04; C07K 1/00, 14/00; C12N 1/21, 15/00, 15/09, 15/63, 15/70; C12P 19/34

## B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

WEST, STN (CAPLUS); DIALOG (MEDLINE, BIOSIS, SCISEARCH, PASCAL)

Terms: att (B?, P?, R?, L?), MCS, POLYLINKER, PLASMID, VECTOR, LOCALIZATION, SIGNAL, TRANSCRIPTION, TERMIN?, TRANSLATION?, ORI, REPLICON, GST, HEXHIST?, THIOREDOX?, CLEAVAGE, SITE?, SPECIF?, DIRECT?, RECOMBIN?, CLON?, INSERT?